Phenology

of

Autumn Gum Moth

Mnesampela privata **(Guenée) (Lepidoptera: Geometridae)**

by

(Stanley)

Zoltan Lukacs

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TABLE OF CONTENTS

Chapter Page

ORIGINALITY OF THESIS

Except where specific acknowledgement is given,
this thesis is my own original work.

Zoltan Lukacs

AUTHORITY OF ACCESS

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ABSTRACT

The autumn gum moth, *Mnesampela privata* (Guenée) (Lepidoptera: Geometridae), is an economically important pest of young eucalypt plantations, particularly those of Tasmanian blue gum, *Eucalyptus globulus* Labill., and shining gum, *E. nitens* (Deane & Maiden) Maiden, which are grown in commercial pulpwood plantations in southern Australia. *M. privata* is also a pest of flooded gum, *E. grandis* W. Hill ex Maiden, plantations grown under irrigation in southern Australia outside that tree's natural range. A major difficulty associated with *M. privata* management is detecting large populations before host trees are severely damaged. Early detection of populations is difficult because outbreaks are sporadic and may be localised, and also because the seasonal occurrence of pest activity varies in some parts of its geographic range. Damaging larval populations on the Australian mainland are largely restricted to the autumn period, April to June, by which time most larvae have pupated. In contrast, pest incidence usually extends from autumn to spring in lowland areas of Tasmania, whereas at altitudes above 500 m ASL in Tasmania, pest incidence usually extends from summer to autumn.

The primary objectives of this study were to identify the underlying mechanisms influencing the phenology of *M. privata* and to identify possible causes of geographic variation in the phenology of the species, thereby increasing our ability to predict the seasonal occurrence of pest activity throughout the distribution of the species.

Laboratory studies found that the phenology of adult *M. privata* was influenced by alternative developmental pathways initiated at specific points in the pupal stage. A diapause or non-diapause pathway was possible early in the pupal stage before adult differentiation began. Pupal diapause was induced by short daylengths and low temperatures experienced during larval development and averted by long daylengths and high temperatures, suggesting that *M. privata* is a long-day species (Type I of Beck's classification). Also possible before adult differentiation began (in non-diapause pupae) was an aestival or non-aestival pathway. Low temperatures led to continued development, high temperatures to aestivation. Unlike diapause, which could only occur before adult differentiation began, aestivation could also occur late in the pupal stage, enabling pharate adults to delay eclosion.

The generation time of *M. privata* was approximately 1268 degree-days (DD) above a developmental threshold of 5°C, consisting of 784 DD for egg-larval development and 484 DD for pupal development. However, the period between oviposition and the fourth instar, or 'time-to-L4' (requiring 390 DD > 5°C) is especially important as it represents the lead time between peak oviposition and the onset of severe damage. At mean daily temperatures of around 12°C, typical of field temperatures experienced by *M. privata* during egg-larval development, time-to-L4 is around 56 days. Hence, large populations of *M. privata* must be detected and controlled within about two months of peak oviposition in order to minimise damage to host trees.

Mnesampela privata females selectively oviposited on expanding terminal shoots, but did not appear to discriminate on the basis of whether or not conspecific eggs were present. It is considered that such oviposition behaviour may lead to overexploitation of resources, but does not fully explain the eruptive population dynamics of the species. Sedentary adult behaviour was observed on two evenings in elevated plantations in Tasmania during summer, suggesting that low temperatures in autumn in those areas might preclude nocturnal reproductive activity.

The incidence of larval feeding activity in Victorian blue gum plantations surveyed at the end of summer was extremely low, suggesting that extensive damage by *M. privata* during summer is highly unlikely outside of Tasmania. Larval feeding damage in the surveyed areas is thus likely to be restricted to the autumn-winter period.

It was concluded that pupal diapause and pupal aestivation are the primary mechanisms regulating the phenology of *M. privata* and that the interaction between these mechanisms and local environmental conditions causes geographic variation in the phenology of the species. The findings of this study will provide a basis upon which reliable predictions may be made in relation to *M. privata* phenology.

LIST OF TABLES

Table Page

Table Page

Table Page

LIST OF FIGURES

Figure Page

Figure Page

Figure Page

Figure Page

Figure Page

1. INTRODUCTION

1.1 GENERAL INTRODUCTION

The autumn gum moth *Mnesampela privata* (Guenée) (Lepidoptera: Geometridae) is an endemic Australian insect that occurs widely across the southern part of the continent (see Fig. 1.7 on p.11). The larval stage of this species has been recorded to feed on 30 *Eucalyptus* (Myrtaceae) species (Table 1.1) and increasing concern over their defoliation of trees in young plantations has elevated their pest status over the last two decades (Abbott, 1993; Bashford, 1993; Neumann, 1993; Phillips, 1993; Farrow *et al.*, 1994; Abbott & Wills, 1996; Neumann & Collett, 1997).

M. privata is most commonly an autumn-winter defoliator and spends summer in the pupal stage. In southeastern mainland Australia, the larval feeding season usually extends from April to June (Farrow, 1996). In southern Tasmania and some areas of south-west Western Australia larvae may develop from autumn-spring and pupate in September (Elliott & Bashford, 1978; Abbott & Wills, 1996) (Fig. 1.1). Its accepted common name, 'autumn gum moth' (AGM), in use since the mid 1960's (Gay, 1966), reflects its univoltine pattern of seasonal development, or ***phenology***, in which adult emergence from over-summering pupae and oviposition usually take place in autumn (i.e. from March to May) (McQuillan, 1985). However, despite its widespread recognition as an autumn-winter defoliator, *M. privata* exhibits marked differences in its phenology in some parts of its geographic range (Fig. 1.1).

The greatest divergence from the typical autumn-winter life cycle of *M. privata* is at elevations above 500 m above sea level (ASL) in Tasmania, where adults usually emerge in December and January (de Little, 1981). Hence, the larval feeding season at higher altitudes in Tasmania runs from summer to autumn and

Table 1.1 *Eucalyptus* species currently recognised as host plants of *Mnesampela privata*. The list includes species reported in Neumann & Collett (1997) and Roberts & Sawtell (1981). Eucalypt classification from Pryor & Johnson (1971) and Brooker & Kleinig (1990).

Scientific Name	Common name
Subgenus Symphyomyrtus	
Section Maidenaria	**Southern blue and swamp gums**
Series Viminales	
E. bridgesiana R.T. Baker	Apple box
E. cinerea F. Muell. ex Benth.	Argyle apple
E. cordata Labill.	Heart-leaved silver gum
E. crenulata Blakely & deBeuzev.	Victorian silver gum
E. cypellocarpa L. Johnson	Mountain grey gum
E. dunnii Maiden	Dunn's white gum
E. globulus Labill.	Tasmanian blue gum
E. gunnii Hook.f.	Cider gum
E. macarthurii Deane & Maiden	Camden woollybutt
E. maidenii F. Muell.	Maiden's gum
E. nitens (Deane & Maiden) Maiden	Shining gum
E. nova-anglica Deane & Maiden	New England peppermint
E. parvifolia Cambage	Small-leaved gum
E. perriniana F. Muell. ex Rodway	Spinning gum
E. rubida Deane & Maiden	Candlebark
E. smithii R.T. Baker	Gully gum
E. viminalis Labill.	Manna gum
Series Ovatae	
E. brookeriana A.M. Gray	Brooker's gum
Section Exsertaria	**Reg gums and tropical white gums**
Series Tereticornes	**Red gums**
E. blakelyi Maiden	Blakely's red gum
E. camaldulensis Dehnh.	River red gum
Section Transversaria	**Eastern blue gums, red mahoganies, grey gums**
Series Salignae	
E. grandis W. Hill ex Maiden	Flooded gum
E. botryoides Smith	Southern mahogany
Section Adnataria	**Ironbarks and boxes**
Series Melliodorae	
E. leucoxylon F. Muell.	Yellow gum
Subgenus Monocalyptus	
Section Renantheria	
Series Obliquae	**Ashes**
E. obliqua L'Hérit.	Messmate stringybark
E. delegatensis R.T. Baker	Alpine ash
Series Piperitae	**Peppermints**
E. amygdalina Labill.	Black peppermint
E. elata Dehnh.	River peppermint
E. risdonii Hook.f.	Risdon peppermint
E. tenuiramis Miq.	Silver peppermint

Abbott (1993) recorded an outbreak of *M. privata* on Marri, *E. calophylla* R. Br.ex Lindl., in Western Australia. The author has collected larvae from ornamental *E. ficifolia* (F. Muell.) in Hobart.

populations spend *winter* in the pupal stage. This contrasts the typical autumn-winter life cycle in lowland areas of southern Tasmania (Elliott & Bashford, 1978) and

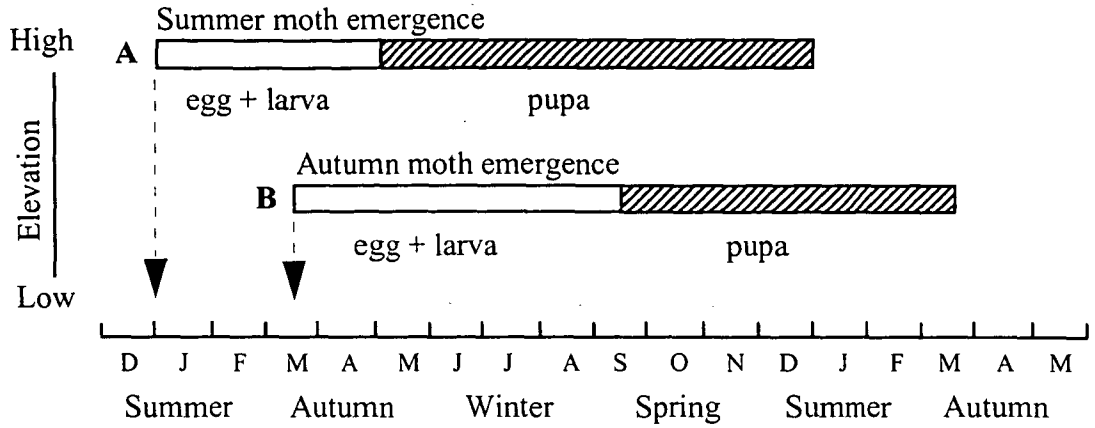


Figure 1.1 Two alternative life cycles exhibited by *Mnesampela privata*. Bar A represents the life cycle at higher elevations (>500 m) in north-western Tasmania which begins in early to mid-summer. Bar B represents the typical life cycle on mainland Australia and in lowland areas of Tasmania, which begins in autumn. Arrows indicate the time that moths begin emerging from pupae. The unshaded areas represent the combined egg and larval stages; the shaded areas represent the pupal stage, which generally lasts between 6 and 10 months.

elsewhere in Australia (Abbott & Wills, 1996; Neumann & Collett, 1997). This pattern of earlier seasonal activity at higher altitudes is common to many species within the genus *Mnesampela* (McQuillan, 1985), but seems particularly well developed in *M. privata*. Consequently, adults can be found in Tasmania at any time from December to June depending on location (Forestry Commission Tasmania, 1977; Elliott & Bashford, 1978; de Little, 1981). Shortly before this study began, a major summer outbreak of *M. privata* in north-western Tasmania defoliated 56 ha of a 175 ha 2 yr old *E. nitens* plantation (Fig. 1.2). The timing of this summer outbreak was very similar to that previously reported in the area by de Little (1981).

On mainland Australia one early report of *M. privata* outside its more common phenology was Froggatt (1923) describing 'thousands of bushes being infested in summer time' at Bendigo (36° 45' S, 144° 17'E; 225 m ASL) in central Victoria. Froggatt's observation suggests a similar life cycle to that reported in elevated areas of north-western Tasmania. However, *M. privata* also occurs above 500 m ASL in south-east Australia and over 1000 m ASL in the Tablelands yet still

Figure 1.2 Aerial view of a *Eucalyptus nitens* plantation in NW Tasmania after defoliation by a major outbreak of *Mnesampela privata* between December 1993 and April 1994. The arrow points to the defoliated area, where the red-brown soil is visible through defoliated tree canopies. Undamaged trees above the arrow were protected by a single application of insecticide in December 1993. (Source: I. Ravenwood, North Eucalypt Technologies).

has the characteristic autumn larval development (R. Farrow pers. comm.). Aside from these observations, geographic variation in the seasonal phenology of *M. privata* has not been investigated and is poorly understood.

1.2 AUTUMN GUM MOTH

1.2.1 Taxonomy and description

The family Geometridae is one of the largest families of moths, with an estimated 20,000 described species (Scoble, 1992), of which 2310 are found in Australia (Nielsen & Common, 1991). *M. privata* is one of seven species within the genus *Mnesampela* Guest (McQuillan, 1985; McQuillan & Edwards, 1996). The genus *Mnesampela* belongs to the subfamily Ennominae (Boarmiinae) which is the largest geometrid subfamily in Australia (Nielsen & Common, 1991). Other economically important native geometrids in this subfamily include *Chlenias* spp. which attack *Eucalyptus* spp., *Pinus* spp., *Cupressus macrocarpa* and apricot trees (Madden & Bashford, 1977a,b) and *Thalaina* spp. which feed on *Acacia* spp. (McQuillan, 1981).

Based on Elliott & Bashford (1978) and McQuillan (1985), general descriptions of all stages of *M. privata* are as follows:

Adults (Fig. 1.3): Adults are about 20 mm long and have a wingspan about twice their body length. The cryptic-coloured forewings are light brown and have irregular, wavy, dark markings, while the hindwings are bright yellow to a light orange. Wings are held roof-wise over the body at rest. Both sexes are very similar in appearance, but they may be distinguished by examination of the wing coupling apparatus. The frenulum of the male consists of one heavily sclerotised, stout spine, while the frenulum of the female consists of several thin bristles.

Eggs (Fig. 1.4): Eggs are laid in batches of up to 300 eggs on the upper and lower surfaces of leaves, with a mean batch size of 75.4 ± 11.2 (s.e.) (Elliott & Bashford, 1978). They are initially a pale yellow to greenish colour, but gradually turn reddish brown as they develop. Eggs are ovoid, slightly flattened dorso-ventrally and measure about 0.9 x 0.6 x 0.4 mm (Elliott & Bashford, 1978; McQuillan, 1985).

Larvae (Fig. 1.5): Larvae are light yellow-brown with darker brown and green markings along the body and have a distinct, light brown head capsule. They can be distinguished by two prominent yellow tubercles on the dorsum of the second abdominal segment. Larvae pass through five instars, growing from neonate larvae just 2-3 mm long to 5th instars 3-4 cm in length.

Pupae (Fig. 1.3): Pupae are shiny, reddish-brown and 10-15 mm in length. The tip of the cremastral region (at the end of the pupal abdomen) has four hooked setae.

1.2.2 Oviposition patterns and larval behaviour

Eggs are laid in batches on expanding juvenile leaves at the tips of lateral branches (Farrow, 1996), mostly on foliage less than five metres in height (Elliott & Bashford, 1978). When population density is high, several egg batches may be laid on a single leaf by one or more females (Fig. 1.4). Early larval instars are highly gregarious and

Figure 1.3 Adults and pupae of *Mnesampela privata*. Wings held roofwise over the body are characteristic of *Mnesampela* adults at rest.

Figure 1.4 *Mnesampela privata* eggs are greenish-yellow and are usually laid in batches. Numerous batches were laid on this single juvenile *Eucalyptus nitens* leaf by late December 1994 in a plantation in NW Tasmania.

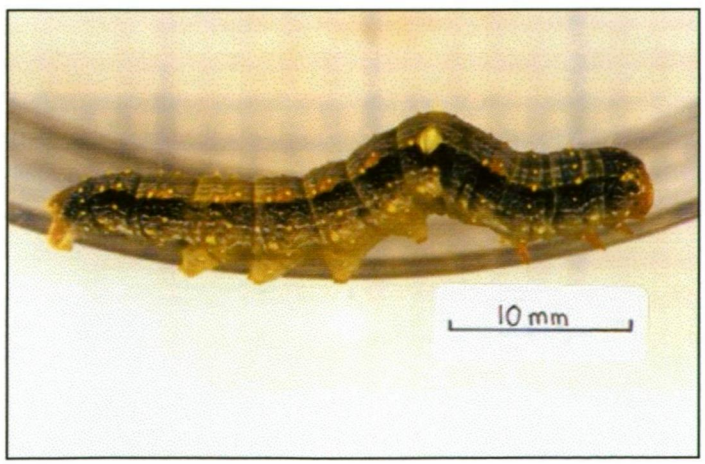
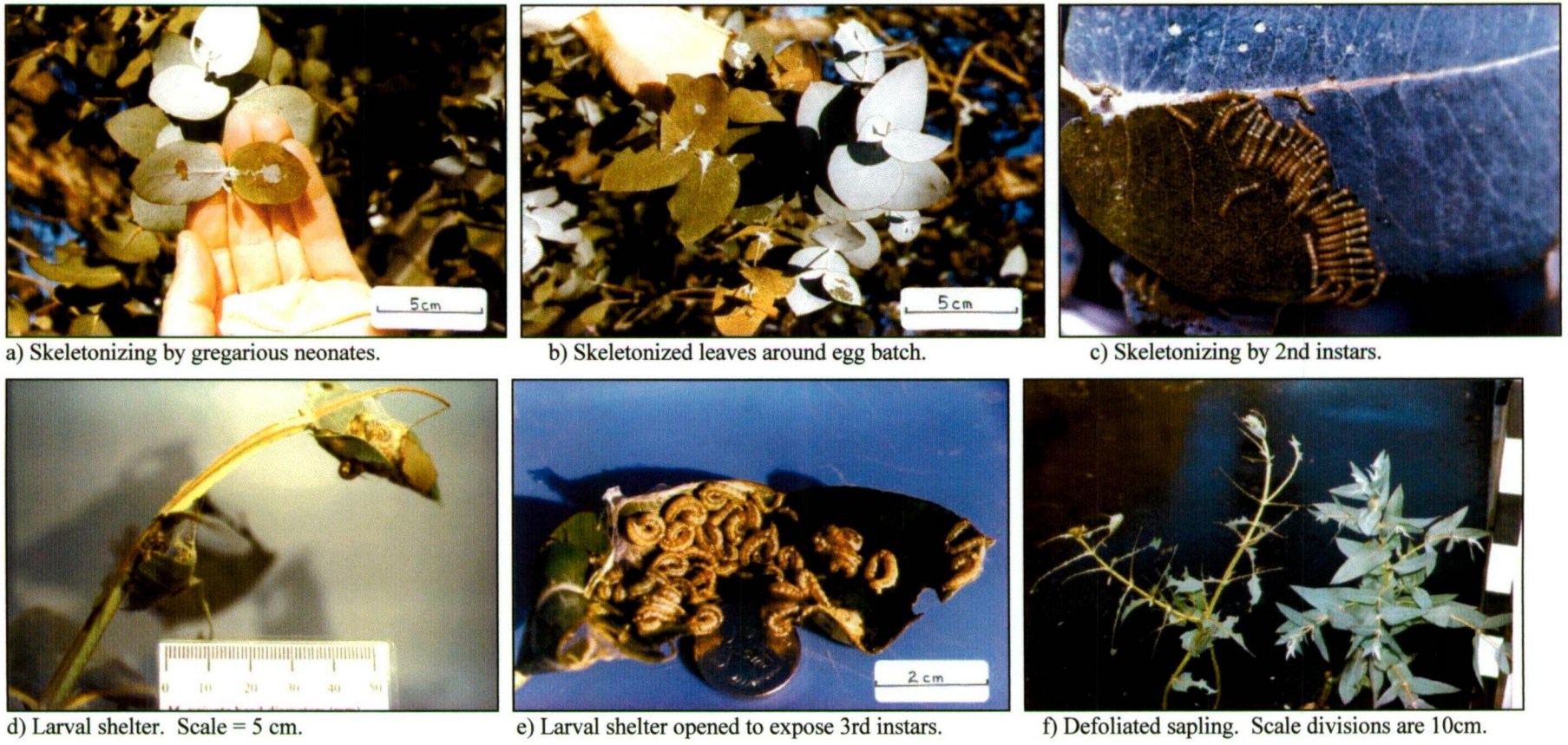


Figure 1.5 Mature, 5th instar *Mnesampela privata* larva showing prominent yellow tubercles on the dorsum of the second abdominal segment.

feed only on the inter-venal tissue of leaves near the empty egg shells (Elliott & Bashford, 1978) (Fig. 1.6a). This method of feeding produces a skeletonizing effect (Fig. 1.6b). Dispersal is very low during the early instars, so most feeding occurs close to the site of hatching (Fig. 1.6b). During the third instar, larvae begin feeding on the leaf edge and webbing terminal leaves together with silk to form larval shelters (Fig. 1.6d,e) in which they hide during the day (Elliott & Bashford, 1978). If disturbed, larvae regurgitate drops of green, eucalypt-scented fluid, possibly as a defence mechanism against predators (Elliott & Bashford, 1978). During the final two instars larvae disperse into surrounding parts of the canopy where they continue feeding, but they may still be found together in smaller groups as all larval instars are somewhat gregarious (McQuillan, 1985). If larval density is too high, the preferred younger shoots may be completely defoliated, forcing larvae to feed on the older and tougher leaves within the canopy. In extreme cases trees may be completely stripped of foliage (Fig. 1.6f), with larvae having to disperse to nearby trees to complete development (Farrow, 1996). When larval development is complete they descend to the ground, burrow into the soil surface beneath their host tree and construct an earthen cocoon from soil particles, silk and body fluids (Elliott & Bashford, 1978). Once the cocoon is spun they rest as a non-feeding pre-pupa for several weeks before pupating inside the cocoon. Damage by *M. privata* larvae is easily recognised by the presence of larval shelters and skeletonized and scalloped leaves (Farrow *et al.*, 1994) and these may be used as indicators of current or recent activity by *M. privata* in an area.



a) Skeletonizing by gregarious neonates. b) Skeletonized leaves around egg batch. c) Skeletonizing by 2nd instars.

d) Larval shelter. Scale = 5 cm. e) Larval shelter opened to expose 3rd instars. f) Defoliated sapling. Scale divisions are 10cm.

Figure 1.6 Feeding damage by *Mnesampela privata* larvae. a) Skeletonizing of a *Eucalyptus cinerea* leaf after eggs hatch. Note the undamaged leaf on the left which has an unhatched egg batch. b) Skeletonized leaves near the original egg batch. The adjacent shoot is virtually undamaged. c) Second instars skeletonising the upper surface of a juvenile *E. globulus* leaf. d) Larval shelter made from leaves webbed together with silk. Larvae are packed tightly together inside the terminal shelter. e) A larval shelter opened up to expose 3rd instar larvae hiding in the shelter during the day. f) Sapling defoliated by larvae. Only the mid-ribs of leaves remain.

1.2.3 Pest status and geographic distribution

M. privata has been recognised as a pest of young eucalypts for almost a century (French, 1900) as it is found most frequently on the juvenile foliage of young trees and is rarely found on the adult foliage of older trees (one exception being *E. grandis*). Young blue gums typically carry juvenile foliage until about age 3-5 years in Tasmania (de Little, 1981; Bashford, 1993), south-western Australia (Abbott, 1993) and Victoria (Neumann, 1993), although good site selection, fertilization and irrigation may hasten the transition to adult foliage (Neumann & Collett, 1997). Consequently, plantations are most frequently attacked by *M. privata* during the first 3-5 years after establishment. Thereafter, they are seldom attacked by *M. privata* but may become hosts for other defoliating insects which feed on the adult foliage type, such as Christmas beetles, *Anoplognathus* spp (Coleoptera: Scarabaeidae) (Carne *et al.*, 1974) and the Tasmanian eucalyptus leaf beetle *Chrysophtharta bimaculata* Olivier (Coleoptera: Chrysomelidae) (Elliott *et al.*, 1992; Bashford, 1993).

In contrast to some pests which may cause damage to plantation areas exceeding one hundred hectares, e.g. the Christmas beetle *Anoplognathus chloropyrus* (Drapiez) (Carne *et al.*, 1974), *M. privata* tends to be a sporadic pest that has localised outbreaks which cause damage to relatively small areas (typically less than 100 ha) such as individual plantations. For example, severe damage by *M. privata* was reported in a 20 ha trial plot near Mt. Gambier in South Australia (Phillips, 1993), in a 5 ha *E. globulus* provenance trial near Tatura in Victoria (Farrow *et al.*, 1994) and was also responsible for a 12% reduction in stem diameter growth of *E. globulus* in one plantation in south-western Australia (Abbott, 1993). However, it may be that such reports of localised damage simply reflect isolated study sites (individual plantations) and not a behavioural trait of the insect. Perhaps

outbreaks are more widespread than reported but go un-noticed due to a lack of monitoring. *M. privata* also contributed to the mortality of 74 of 80 trees in a eucalypt trial planting on the Northern Tablelands of N.S.W. (Roberts & Sawtell, 1981). The latter led Roberts and Sawtell (1981) to conclude that *M. privata* was by far the most important pest of *E. bicostata*, *E. blakelyi* and *E. gunnii* in their trial planting during the first three years after planting. Since damage by *M. privata* tends to occur in relatively small areas, it is more difficult to monitor for outbreaks of this species than, for example, Christmas beetles which cause damage over broader areas and are therefore more apparent. As a consequence of such monitoring difficulties, damage by *M. privata* is often found too late for effective action (i.e. insecticide application) to be taken (Farrow *et al.*, 1994).

Among the southern blue gum species most frequently attacked, two closely related species are widely used in commercial forest plantations in southern Australia. Shining gum, *E. nitens*, and Tasmanian blue gum, *E. globulus*, both have a high growth rate and good fibre quality (Tibbits, 1986; Beadle *et al.*, 1989; Williams, *et al.* 1995) and are preferred by the pulpwood industry in southern Australia which is expanding into native hardwoods (Table 1.2). *E. globulus* is prominent in plantings in south-west Western Australia (Abbott, 1993), both species

Table 1.2 Approximate size of the blue gum plantation estate across southern Australia in 1993 and the total eucalypt plantation estate in the same states in 1995. (1993 figures from Bashford, 1993; Neumann, 1993; Abbott, 1993; Phillips, 1993. 1995 Figures from ABARE, 1998).

State	Blue gums 1993 (ha)	Approx. Annual Planting rate (ha)	Eucalypt total 1995 (ha)	Estimated 1998 (ha)
Tasmania	41 000	5 000	62 018	65 000
Victoria	20 000	na	19 307	20 000
Western Australia	10 000	[a] 20 000	42 042	[a] 80 000
South Australia	2 000	500	[b] 1 038	4 500
Total	73 000	25 500	124 405	169 500

[a] Pers. comm. R. Floyd, CSIRO Entomology, Canberra. [b] Indicates a net loss after harvesting.

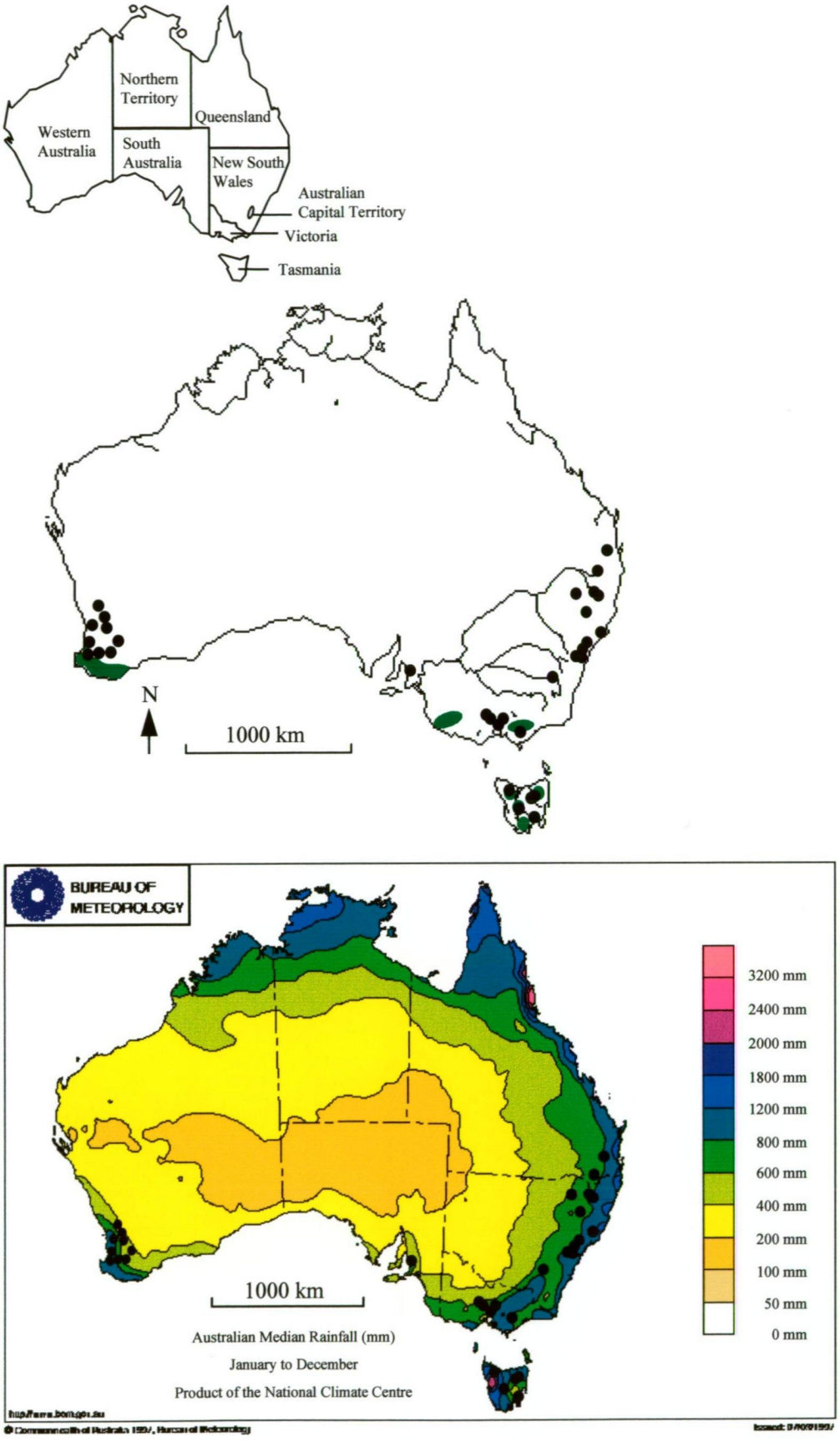


Figure 1.7 The distribution of *Mnesampela privata* in Australia (black dots) based on collected adult specimens (from McQuillan, 1985). Top map shows state borders; green areas in the middle map indicate where plantations of *Eucalyptus globulus* (mainland) and *E. nitens* (Tasmania) are currently being established. Bottom map shows the distribution of *M. privata* in relation to median annual rainfall. Source of rainfall map: Australian Bureau of Meteorology (1998).

are planted in Victoria (Neumann, 1993) and *E. nitens* is preferred in Tasmania, particularly at higher altitudes because of its superior frost tolerance (Eldridge *et al.*, 1993; Williams *et al.*, 1995). Blue gums are being planted either alongside (Neumann, 1993) or instead of (Tibbits, 1986) members of the ash group of eucalypts (Series Obliquae of Pryor & Johnson, 1971) (e.g. *E. delegatensis*, *E. obliqua* and *E. regnans* F. Muell) which were historically used by the timber industry. Apart from *E. delegatensis* and *E. obliqua*, ashes are not defoliated by *M. privata*. This shift away from ashes towards blue gums has contributed significantly to the increased pest status of *M. privata* over the past two decades. Another reason is that *M. privata* has become a major pest of irrigated plantations of *E. grandis* in the Murray Valley (Victoria) and elsewhere (R. Farrow pers. comm.).

By 1993, the blue gum plantation estate established in southern Australia was approximately 73 000 ha (Table 1.2). The annual planting rate is presently about 25 000 ha, with 80 percent of this being in Western Australia, where the aim is for 100 000 ha of blue gum plantations by the year 2000 (Abbott, 1993). However, the annual planting rate is likely to increase further as a result of the Australian government's decision in 1996 to treble Australia's forest plantation estate by the year 2020 (Australian Bureau of Statistics, 1997). Eucalypt plantations are established in areas where the annual rainfall usually exceeds 600 mm (Abbott, 1993; Neumann, 1993) and since *M. privata* occurs in open forest where annual rainfall usually exceeds 500 mm (McQuillan, 1985) (but see below), the areas planted with *E. globulus* and *E. nitens* are largely within the distribution of *M. privata* (Fig. 1.7). It must be noted here that the distribution of *M. privata* extends further inland (into drier areas) than reported by McQuillan (1985). *M. privata* has caused total defoliation of irrigated plantations of *E. grandis* at Mildura (34° 14'S, 142° 05' E, 50m ASL, 297mm mean annual rainfall) (Z. Lukacs pers. obs.).

1.3 FACTORS CONTROLLING INSECT PHENOLOGY

1.3.1 Seasonal variation

Insects living in temperate climates usually experience regular, seasonal variations in their abiotic environment, including temperature, daylength and rainfall patterns, and with their biotic environment such as host-plant phenology, food supply, natural enemies and competitors (Tauber *et al.*, 1984; Danks, 1994a; Bradford & Roff, 1997). Because of such environmental variation, many insects in temperate regions are only active during part of the year when conditions are most favourable for reproduction and development (Dingle, 1986; Topp, 1994; Bradford & Roff, 1997). For instance, the majority of insects in northern hemisphere temperate zones are active during the warm season (Sauer *et al.*, 1986) and have evolved various life-history strategies (e.g. dormancy or migration) which enable them to survive the unfavourable winter months and synchronise their activity with favourable seasonal conditions (Dingle, 1978; Dingle, 1986; Bradshaw, 1986; Sauer *et al.*, 1986; Danks, 1994a; Bradford & Roff, 1997). However in Australia, winters are less severe and summer drought is arguably the most important factor influencing insect life cycles. In Australia it is therefore not unusual for some insects to breed in autumn, winter and spring (March-November). For example the Bogong moth, *Agrotis infusa* (Boisd.) (Lepidoptera: Noctuidae) breeds in winter and aestivates as an adult from November to February (Common, 1954). Two other noctuid species, *Helicoverpa armigera* (Hübner) and *H. punctigera* (Wallengren), breed in winter in inland areas of Australia before migrating to eastern coastal areas where they breed in summer as well (Zalucki, *et al.*, 1986; Zalucki *et al.*, 1994). Meanwhile, adverse temperature and moisture conditions in both summer and winter usually restrict development of the active stages of the Australian plague locust, *Chortoicetes terminifera* Walker (Orthoptera: Acrididae), to spring and autumn (Wardhaugh, 1986).

1.3.2 Diapause

Of all the mechanisms which enable insects to synchronise their life cycles to favourable seasonal conditions, diapause is the most important (Danilevskii, 1961; Beck, 1968; Waldbauer, 1978; Tauber *et al.*, 1984; Denlinger, 1985; Bradshaw, 1986; Wardhaugh, 1986; Grüner & Masaki, 1994). Diapause is defined by Beck (1968) as 'a genetically determined state of suppressed development, the manifestation of which may be induced by environmental factors'. Generally, diapause arrests normal development and prevents a particular life-history stage from entering adverse conditions which it could not easily survive, such as extreme temperatures, dry conditions and during periods when food is scarce (Beck, 1968; Tauber *et al.*, 1984; Denlinger, 1985; Gillott, 1995; Bradford & Roff, 1997; Johnsen *et al.*, 1997). Once the adverse conditions pass, insect development resumes in synchrony with favourable conditions and also with other members of the species.

Diapause is induced by token stimuli in the environment which, although not detrimental themselves, reliably predict the approach of unfavorable conditions (Beck, 1968; Tauber *et al.*, 1984; Denlinger, 1985). Daylength in particular, is a widely used token stimulus for diapause induction because it is a reliable predictor of seasonal change (Denlinger, 1985), but temperature may interact with daylength to modify the diapause decision in many species, sometimes over-riding the diapause decision altogether (Beck, 1968). Other factors such as moisture and diet may be important in some insects, but have little or no influence on diapause induction in most species (Beck, 1968; Gillott, 1995).

In contrast to simple quiescence which occurs in direct response to adverse conditions, diapause *anticipates* the arrival of adverse conditions and prevents further development even under favorable conditions. As a consequence of this developmental interruption, diapausing individuals can remain inactive for months

while non-diapause individuals held under identical conditions complete their development without delay (Howe, 1967). For example, non-diapause pupae of *Helicoverpa punctigera* require 10-12 days for adult differentiation at 28°C, but pupal duration may exceed 200 days at 28°C because of a pupal diapause that lasts up to six months (Cullen & Browning, 1978).

Diapause is found in all developmental stages of the insect life-cycle, but in most species it occurs at a particular developmental stage which is characteristic of the species (Tauber *et al.*, 1984; Denlinger, 1985). For instance, diapause in the winter moth, *Operophtera brumata* (L.) (Lepidoptera: Geometridae), occurs in the egg stage and it is necessary to determine when diapause ends and post-diapause development begins before degree-day summation can begin in a larval eclosion model for that species (Kimberling & Miller, 1988). However, there is some disagreement in the literature as to whether developmental interruptions in the egg stage of winter moth are caused by diapause or quiescence (Holliday, 1985). Some insects can diapause in more than one stage in their life cycle, but this ability is very rare (Tauber *et al.*, 1984; Denlinger, 1985; Bradshaw, 1994; Gillott, 1995).

Once diapause has ended, insects regain the capacity to resume development and may complete develop without further interruption (as in *H. punctigera* above). However, post-diapause development may be interrupted by a period of quiescence in response to unfavorable conditions, such as continuing low or high temperatures (Tauber *et al.*, 1984). In this situation, development will only be completed after the adverse conditions pass.

1.3.3 Geographic variation

In addition to seasonal variation in the environment, insect species with broad geographic ranges must be able to cope with local environmental conditions. To overcome this problem, considerable flexibility exists in life-history traits of many insect species which allows the local life-history to be shaped by natural selection (Dingle, 1986; Danks, 1994; Gomi & Takeda, 1996). Some examples of such flexible life-history traits include the developmental threshold (Campbell *et al.*, 1974) and rate of development (Holliday, 1985; Gomi & Takeda, 1996), diapause induction and length (Ch. 9 of Beck, 1968; Gomi & Takeda, 1996) and body size (reviewed in Gomi & Takeda, 1996). Generally, natural selection favors traits which are advantageous to an organism's survival (Begon *et al.*, 1986). Therefore, life-history strategies which turn out to be advantageous to survival in a particular habitat are likely to be favoured by natural selection and adopted in that local habitat. However, as noted by Begon *et al.* (1986) (p. 501), "the most that natural selection can do... is to favour the life-history that is best (not 'perfectly') suited... [to] an organism's environment".

The ability to adapt to local conditions has meant that many insect species have developed markedly different life cycles in different geographic areas. For example, the phenology of adult emergence differs markedly with altitude in the lycaenid butterfly, *Euphilotes enoptes* (Boisduval) (Lepidoptera: Lycaenidae) (Peterson, 1995) and is closely associated with the flowering phenology of its host plant. Peterson (1995) also found evidence that substantial gene exchange occurred among populations along the altitudinal gradient, which suggested that the phenological differences had no genetic basis and were mainly due to the different environments. The latter is an example of 'phenotypic plasticity', a term used to

describe a situation where different phenotypes occur in response to environmental rather than genetic differences (Nylin, 1994; Via, 1994).

On a larger geographic scale, the voltinism and seasonal pattern of adult emergence of the cabbage moth *Mamestra brassicae* L. (Lepidoptera: Noctuidae) varied with latitude in Japan (Grüner & Masaki, 1994). Firstly, voltinism ranged from one summer generation in northern Japan to three generations (spring, summer and autumn) in southern Japan. Secondly, adults emerged from overwintering pupae progressively earlier at lower latitudes where the warmer climate early in the season accelerated development during the pupal stage. However, the opposite was true during autumn, when adults emerged progressively later at lower latitudes because adversely high temperatures late in the season retarded pupal development. The latitudinal differences in adult emergence patterns of *M. brassicae* were largely due to phenotypic plasticity as alternative forms of pupal diapause (summer diapause, winter diapause and non-diapause) occurred in direct response to different local environments. At lower latitudes, long daylengths and higher temperatures during summer induced a period of summer diapause in many individuals during the pupal stage, whereas at higher latitudes summer diapause was uncommon or absent because of the cooler climate. However, different populations had different daylength thresholds for diapause induction, which suggested a genetic component as well. Thus, geographic variation in phenology may be difficult to interpret because of the combined effects of phenotypic plasticity and genetic differentiation (e.g. Day, 1997).

1.3.4 Causes of phenological variability in other geometrid species

The following discussion examines factors controlling lepidopteran phenologies including those of the winter moth, *O. brumata*, (reviewed by Holliday, 1985) and the autumnal moth, *Epirrita autumnata* (Borkhausen) (Lepidoptera: Geometridae).

Operophtera brumata and *E. autumnata* are two northern hemisphere geometrids which share *M. privata*'s unusual habit (see Coulson & Witter, 1984) of emerging from pupae and ovipositing during the cooler months of the year and the winter moth also demonstrates considerable geographic variation in the timing of adult emergence (Wylie, 1960; Holliday, 1985). Winter moth adults emerge over a two-month period at any one locality but, as the exact time of the two month emergence period varies with locality, the adult emergence period of the species extends over a five month period from September to January (autumn and winter in the northern hemisphere) (Wylie, 1960; Holliday, 1985). Adult emergence is earlier at higher latitudes than at lower latitudes and is also earlier at higher altitudes than in adjacent lowland areas (Holliday, 1985). The latter in particular is analogous to the earlier emergence of *M. privata* adults at higher altitudes in Tasmania (Fig. 1.1).

Climate and host plant phenology are the major factors maintaining the phenology of winter moth (Holliday, 1985). Climatic extremes during summer and late in winter restrict the period of moth emergence and oviposition to autumn and early winter (i.e. between summer heat and winter cold), although avoidance of predatory beetles which are active during the warmer months is suggested to be a secondary factor opposing emergence before autumn (Holliday, 1985; Topp & Kirsten, 1991). Generally, emergence of winter moth in summer is prevented because summer temperatures are above-optimal and slow the rate of pupal development (Holliday, 1985; Topp & Kirsten, 1991). However, winter moth adult activity must be completed before severe winter weather sets in, so the emergence period cannot be too late (Holliday, 1985).

The phenology of egg hatching and larval development of *O. brumata* is influenced by host-plant phenology and by temperature constraints as larvae suffer

from exposure to high temperatures in summer (Holliday, 1985). Eggs of both *E. autumnata* and *O. brumata* are laid in protected places on branches and trunks of host trees during autumn and winter, but do not hatch until the following spring (Tammaru *et al.*, 1995; Van Dongen *et al.*, 1997). Eggs hatch early in the season in synchrony with bud-burst of their deciduous host trees. Host foliage quality for larvae declines steadily as leaves mature and winter moth larvae fed on young oak leaves weighed about three times as much as larvae fed on more mature oak leaves (Feeny, 1970). Because of the tight association between the phenology of winter moth and its host plants, the exact timing of the three week hatching period in an area varies depending on the phenology of local host plants. Thus, eggs hatch over a three week period in any one locality, but hatch throughout spring (March to May) across the entire distribution of the species (Holliday, 1985). However, larvae that hatch too early, i.e. before bud-burst, risk starvation (Holliday, 1985). Since the phenology and ecology of *E. autumnata* is very similar to *O. brumata* (Peterson & Nilssen, 1996), these same factors are likely to influence the phenology of *E. autumnata*. Thus, although seasonal and geographic differences in climate may directly influence insect phenology, seasonal and geographic variation in host plant phenology may also need to be considered when attempting to explain phenological variability of a herbivorous insect. The fact that eucalypts are evergreen instead of deciduous does not mean that host plant phenology will be unimportant for *M. privata* because, like winter moth feeding on oak, seasonal changes in leaf quality may constrain larval phenology to certain periods of the year, such as when leaves are young and expanding.

1.4 PHENOLOGY MODELS AND INTEGRATED PEST MANAGEMENT

A good appreciation and understanding of population phenology is essential for efficient management of insect pests, particularly in predicting the timing of the main

phases of insect life cycles: reproduction, growth and development, dormancy and migration or dispersal (Tauber & Tauber, 1978; Tauber *et al.*, 1984). Since temperature is the dominant environmental factor influencing insect development and growth in temperate latitudes (Howe, 1967; Campbell, *et al.*, 1974; Logan *et al.*, 1976; Gilbert, 1984b, 1988; Gordon, 1984) and is easily measured, phenology models relating insect development to temperature can be integral parts of management strategies for insect pests as they assist with pest sampling and control decisions. For example, control of the codling moth, *Cydia pomonella* L. (Tortricidae) by pesticide application is timed by using a phenology model which predicts the time of egg hatch after the sustained capture of moths in pheromone traps (Beers & Brunner, 1992). Similarly, larval eclosion models for gypsy moth, *Lymantria dispar* (L.) (Lymantriidae) (Johnson *et al.*, 1983) and the winter moth, *O. brumata* (Kimberling & Miller, 1988) predict the timing of egg hatch and first instar dispersal, which assists in the timing of the use of pesticides or the release of biological control agents. A larval model for *L. dispar* predicts when suppression of early instars may be needed, or alternatively, when to release pupal parasites after larvae have finished feeding and pupated (Casagrande *et al.*, 1987). This larval model has been successfully used to time applications of *Bacillus thuringiensis* Berliner in the United States (Casagrande *et al.*, 1987). A pupal model for gypsy moth predicts when adults will emerge and therefore, when to begin pheromone trapping of males in population sampling programs (Casagrande *et al.*, 1987), while a pupal model for the Mexican rice borer, *Eoreuma loftini* (Dyar) (Pyralidae) predicts the best time for mating disruption of that pest by synthetic pheromones (Spurgeon *et al.*, 1995). A common theme in these examples is that phenology models guide the use of *other* elements in an overall management strategy. Hence, although the objective of this thesis is to develop a better understanding of *M. privata* phenology,

it is important to remember that any information gathered will need to be integrated into an overall pest management strategy for the species, which remains to be developed.

Finally, good phenological knowledge of a pest insect can be valuable when attempting to explain unexpected phenological differences between areas. A shift in voltinism by the fall webworm, *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) within 50 years of accidental introduction to Japan was attributed to local changes in the insect's development rate and critical photoperiod for diapause induction (Gomi & Takeda (1996). Should *M. privata* be accidentally introduced into countries where eucalypts are grown as exotics in large plantations, knowledge of *M. privata* phenology would be essential in AGM pest management, particularly for forecasting the most likely phenological pattern (i.e. summer or winter pest incidence) in newly-inhabited areas.

1.5 AIMS AND OBJECTIVES

Despite *M. privata*'s potential as a major defoliator of young eucalypt plantations, there is currently insufficient knowledge about this insect to develop sustainable pest management strategies. In particular, the lack of detailed phenological information on *M. privata* increasingly represented a significant gap in the knowledge required to efficiently manage this insect pest. Accordingly, based on what was known about (i) the problem, (ii) *M. privata*, (iii) insect phenology and (iv) what is required for pest management, the primary objectives of the study were to identify the underlying mechanisms influencing the phenology of *M. privata* and to identify possible causes of geographic variation in the phenology of the species.

Given that many insect species may exhibit different life-histories in different localities because of climatic differences, the hypothesis tested in this thesis was that

geographic variation in the phenology of *M. privata* is caused by different life-history strategies in response to different climatic environments. Hence, the thesis must show that different phenologies of *M. privata* are (a) associated with, and (b) caused by, different climatic environments.

Adult phenology and generation time were hypothesised to be the key factors likely to influence *M. privata* phenology and were therefore chosen as the two main areas of investigation in this study. The research program begins by investigating adult emergence patterns following larval development in the field in order to identify possible key factors influencing the phenology of adult *M. privata* (Chapter 2). The next step was to test whether adult phenology could be manipulated by changing the environment under which insects developed. To achieve this, *M. privata* was reared from eggs to the pupal stage under different temperature and lighting conditions in the laboratory and then to adult emergence at a standard temperature of 15°C. A secondary consideration when rearing *M. privata* in the laboratory was to relate developmental times of all life-history stages to temperature so that it would be possible to predict the developmental time of an entire generation, or any part of it, in any area from local average temperatures. Hence, the temperature response of *M. privata* is investigated in Chapter 3 and is followed immediately by an examination of adult emergence patterns at 15°C following larval development at various temperatures and daylengths (Chapter 4). By comparing adult emergence patterns after larval development in the field and laboratory, I hoped to associate alternative adult phenologies with different larval rearing environments.

Following the three experimental chapters, field observations of *M. privata* are presented and interpreted in relation to the laboratory findings. Early-season activity (adult phenology and oviposition) is investigated in the second year of a

major outbreak at a summer-emergence site in NW Tasmania (Chapter 5), while Chapter 6 puts the early life-cycle at high altitudes in Tasmania into perspective with what typically happens during summer on mainland Australia by investigating the level of summer larval activity present in Victorian blue gum plantations. The final chapter (Chapter 7) draws general conclusions about factors causing geographic variation in the phenology of *M. privata* and discusses implications for forest lepidopteran biology and ecology in general, for which this study has important implications. First, phenological variability of *M. privata* in Tasmania is an ideal system in which to study factors causing alternative life-history strategies in a single species. Second, since relatively few insects develop during winter, this study represented an ideal opportunity to study factors maintaining an uncommon life-history pattern.

Finally, the possibility that genetic differences occurred between geographic populations was also investigated, first by comparing temperature responses of different populations (Chapter 3), then by allozyme electrophoresis. However, since early results of the allozyme study were not promising (see Appendix C), that particular area of investigation was not pursued beyond the pilot stage and does not appear in the thesis.

2. ADULT PHENOLOGY OF *MNESAMPELA PRIVATA*

2.1 INTRODUCTION

Phenological knowledge is a basic requirement for insect pest management (Tauber & Tauber, 1978; Tauber et al., 1984; Pedigo, 1989). Basic phenological knowledge makes it easier to predict when seasonal activity will begin and when the damaging phase of pest populations will occur. *Mnesampela privata* is a univoltine lepidopteran pest that causes over 95% of damage during the final two instars of its five instar larval stage (Phillips, 1996). Therefore, high densities of adult moths, eggs or early larval stages which cause only minor skeletonizing damage provide advance warning that damage may occur as the season progresses. Large populations must be detected and suppressed before larvae reach the fourth instar if damage is to be prevented. It is therefore unfortunate that detection of large populations early in the season is a major difficulty associated with *M. privata* management, as damage by *M. privata* is often found too late for effective action to be taken (Farrow *et al.*, 1994; Floyd *et al.*, 1994). Contributing to this difficulty are the sporadic and sometimes localised nature of *M. privata* outbreaks and a lack of detailed phenological information on the species.

Although *M. privata* is widely distributed across southern Australia, its annual life-cycle is relatively uniform throughout its distribution. The life-cycle in most areas begins with an autumn adult emergence (from pupae), autumn oviposition and subsequent larval development during late autumn, winter and spring (Elliott & Bashford, 1978; Farrow, 1996). The only routinely observed departure from this typical cool-season phenology occurs in Tasmania, but only at altitudes above 500 m ASL. At these altitudes in Tasmania, adults usually emerge and oviposit during December and January (de Little, 1981). This enables the larval phase to be completed by April-May (see Fig. 1.2), at a time eggs are still being laid in most

other areas of the species' distribution. In lowland areas of Tasmania, *M. privata* phenology follows the common autumn-winter pattern. Hence, while phenological information on *M. privata* is primarily needed to make it easier to determine the best time in autumn to carry out monitoring operations for most areas, it is also needed to explain the earlier life cycle at high altitudes in Tasmania.

Since previous studies of *M. privata* described geographic variation in adult phenology (Elliott & Bashford, 1978, de Little, 1981), the timing of adult emergence from pupation was originally hypothesized to be the critical event influencing local phenology (Fig. 2.1). The pupal stage of *M. privata* usually lasts 5-7 months in the field (Elliott & Bashford, 1978; Neumann, 1993) and had a mean duration of 187.7 ± 8.9 (SE) days in the laboratory (Elliott & Bashford, 1978). Little else is known about this stage of the life-cycle.

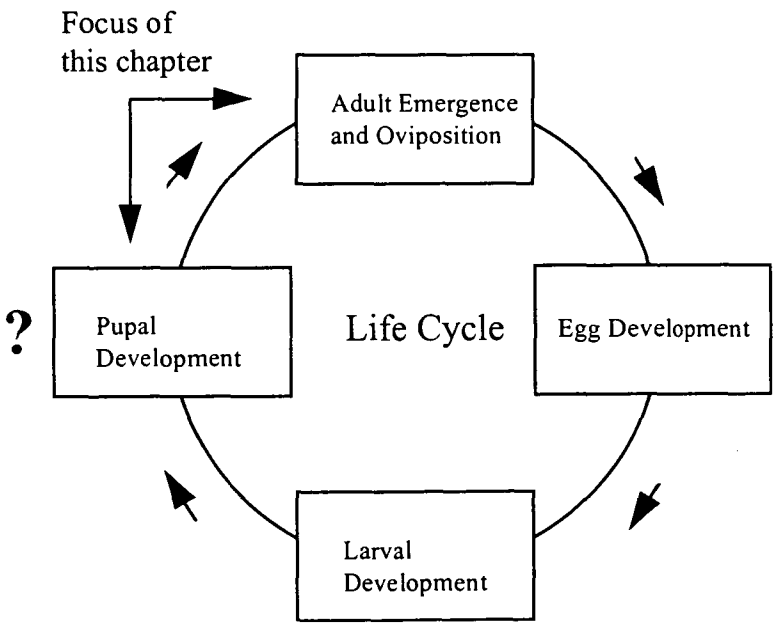


Figure 2.1 Schematic diagram showing the phases in the life-cycle of *M. privata*. Adult emergence was hypothesised to be the critical event in the life-cycle. This chapter investigates what happens between pupation and adult emergence.

A necessary first step towards understanding the insect's phenology was to develop a better understanding of what happens between pupation and adult emergence. To address this aim, adult emergence patterns of *M. privata* were investigated using laboratory collections of pupae reared from field-collected larvae. This method of investigation was necessary after early attempts to harvest pupae from the field for experimental purposes were unsuccessful. By investigating what happened between pupation and adult emergence, I hoped to identify key factors influencing adult phenology. Finally, because the phenologies of *M. privata* expressed in Tasmania appear to be representative of the species' entire distribution, identifying the causes of geographic variation in phenology in Tasmania could have wider implications for mainland populations.

2.2 METHODS

2.2.1 Localities where M. privata *larvae were collected*

Field surveys of *M. privata* distribution in commercial *E. nitens* plantations in north-west, north-east, central and southern Tasmania were carried out during the first year of the study to identify sources of insect material for laboratory experiments. Juvenile *E. nitens* plantations in NW and central Tasmania were subsequently chosen as field collection sites because moths, eggs or larvae were present at the time the areas were surveyed, or because defoliation indicated that *M. privata* populations were established in the areas and might be expected again in following seasons. Plantations in NW Tasmania were situated within a privately owned tree farm named *Surrey Hills*, while plantations in central Tasmania were situated near Tarraleah (Fig. 2.2). Insects were also collected in the Hobart metropolitan region from *E. cinerea* and other ornamental eucalypts growing in open parkland at Cornelian Bay (5km from the city centre), from natural *E. globulus* regeneration on roadsides and from a small (~2 ha) mixed planting of juvenile *E. nitens* and *E. globulus* at

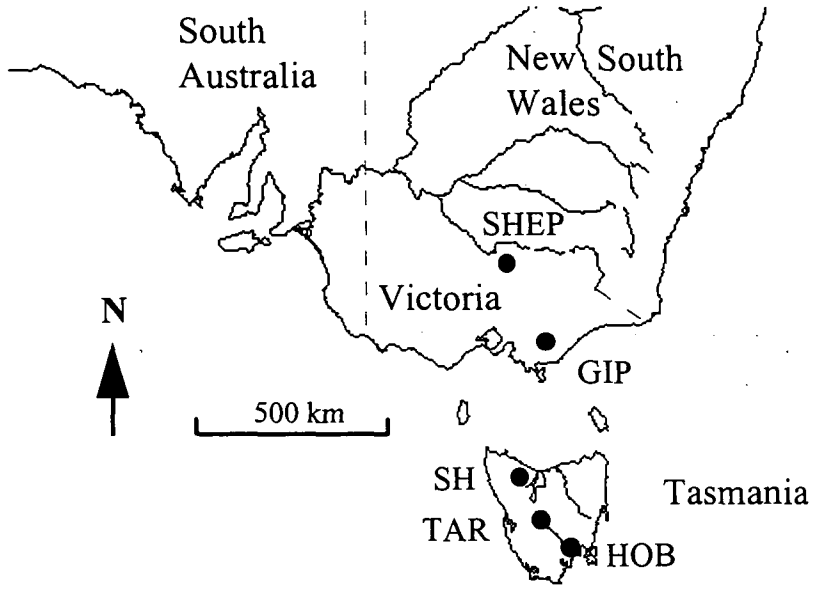


Figure 2.2 Localities where 5th instar *M. privata* larvae were collected during the study. Tasmanian localities: SH = *Surrey Hills*; TAR = Tarraleah; HOB = Hobart. Victorian localities: SHEP = Shepparton region; GIP = Gippsland region. Larvae were collected at *Surrey Hills* and Hobart in 1995 and from all localities in 1996.

Table 2.1 Climatic details of the localities where 5th instar *M. privata* larvae were collected.

					Mean Daily Temperature (°C)			
					January		July	
Locality	Latitude	Longitude	Elevation (m ASL)	Mean Annual Rainfall (mm)	Min.	Max.	Min.	Max.
Shepparton	36° 23'S	145° 24'E	114	496	13.8	29.0	2.9	12.8
Gippsland [a]	38° 12'S	146° 24'E	155	896	12.1	25.4	3.8	12.8
Surrey Hills [b]	41° 27'S	145° 32'E	612	2204	6.3	17.6	0.8	7.2
Tarraleah	42° 18'S	146° 27'E	589	1175	6.7	18.6	-0.1	8.1
Hobart	42° 53'S	147° 20'E	50	624	11.8	21.5	4.5	11.6

[a] Meteorological information for the Gippsland region is for Yallourn. [b] Meteorological information for *Surrey Hills* is based on the nearest meteorological station of Waratah which is 18 km from the main collection areas. (Source: Australian Bureau of Meteorology, 1998).

Sorell (42° 47'S, 147° 34'E) approximately 30 km east of Hobart (Fig. 2.2). In the second year of the study, larvae were also collected from *E. globulus* plantations in two areas of Victoria (Fig. 2.2). Climatic details for each collection area are presented in Table 2.1.

2.2.2 Pilot study (1995)

Eight groups of fifth (final) instar larvae were collected at *Surrey Hills* and Hobart between 19th February and 31st October (see Table 2.2). After collection, larvae were incubated at 16.5°C (16L:8D) until pupation, except for two groups collected in July that were reared to pupation on a laboratory bench under room conditions of approximately 22°C and 8L:16D. Pupae from 19.Feb.95 were kept in plastic rearing cups (43 mm diameter, 28 mm deep) and covered with soil from the collection site to mimic natural conditions. However, remaining groups of pupae were held in inverted vial lids (25 mm diameter, 10 mm deep) and partially covered with vermiculite (Fig. 2.3). Pupation dates were recorded for each individual. After pupation, pupae were held in 500 ml transparent plastic boxes (175 x 115 x 35 mm) at 16.5°C (16L:8D) or at 22°C on the laboratory bench (the July groups) and checked weekly for adult eclosion. Pupae were mist-sprayed weekly to reduce desiccation. Pupae from 19.Feb.95 and 5.Apr.95 were transferred from 16.5 to 10°C on 13th April and 16th May 1995 respectively after unexpected early emergence of adults in the 19.Feb.95 group (see results). From October 1995 onwards, pupae were

Figure 2.3 *M. privata* pupae were usually held individually in labeled, inverted vial lids and partially covered with vermiculite.

examined every 1-2 weeks under a stereo-microscope to identify visible changes in pupal appearance as pupae developed. After all pupae in the eight groups completed development, trends in pupal duration and adult emergence were examined in relation to larval collection time, geographic origin and incubation temperature. Linear regressions explained part of the data well and were used to quantify the relationship between larval collection time and both pupal duration and adult emergence time.

2.2.3 Main study (1996)

Fourteen groups of fifth instar larvae were collected from *Surrey Hills*, Hobart and Tarraleah between 15th February and 18th November 1996 (see Table 2.3). An additional group of fifth instars was collected from blue gum plantations in Victoria from 5-8th March 1996. To standardise the measurement of pupal duration in relation to temperature, groups of pupae were held at either 11.5°C or 15°C following pupation at these same temperatures. However, thirty-one pupae from the 14 groups were transferred to higher temperatures once pupal development began in order to assess temperature effects on pupal development. The onset of pupal development was determined using pupal characteristics identified in the pilot study (see results). Pupae transferred to higher temperatures were not used to assess pupal duration at 11.5 and 15°C. Pupal development times at higher temperatures are presented in Chapter 3.

Because of unexpected early emergence of some adults in the pilot study, laboratory rearing conditions following larval collection were deliberately manipulated at 15°C in order to identify possible causes of early adult eclosion. The different rearing conditions at 15°C are described below. Unless stated, pupae were held in inverted vial lids inside 500 ml plastic boxes as described previously.

15°C (16L:8D): All individuals in four groups collected between 15th February and 12th March 1996, and in one group amalgamated from several small collections from Hobart between 4th August and 18th November 1996, were held at 15°C (16L:8D) after initial larval collection. Despite a 1.5°C difference in temperature, these five groups were used as controls for direct comparison with pupae held at 16.5°C (16L:8D) in the pilot study.

Influence of Daylength

The possibility that exposure to a 16hr daylength, either between L5 collection and pupation or during the pupal stage itself, may have caused early adult eclosion in the laboratory was investigated in three trials (Table 2.2), each using a separate group of larvae collected in Hobart between June and October.

Table 2.2 Summary of the three daylength trials used to test the general hypothesis that exposure to a long daylength during the fifth instar or the pupal stage may cause early adult eclosion in the laboratory.

Trial	Hypothesis	Treatment
1	Exposure to a long daylength during the fifth instar averts pupal diapause and causes adult eclosion within 40-60 days of pupation.	Fifth instars reared to pupation at 16L:8D and 8L:16D. Pupae then reared to adult eclosion in darkness.
2	Exposure to a long daylength during the pupal stage averts pupal diapause and causes adult eclosion within 40-60 days of pupation.	Prepupae placed at pupation environments of 16L:8D (Naked-Lit), 0L:24D (Naked-Dark) and into a container of soil (Soil treatment).
3	As for Trial 2.	Prepupae placed at pupation environments of 16L:8D (Naked-Lit), 0L:24D (Naked-Dark), a container of soil (Soil-Lit) and a container of soil wrapped in foil (Soil-Dark).

Trial 1: This trial was designed to test the hypothesis that long-day exposure between L5 collection and pupation caused adult eclosion within 40-60 days of pupation. Seventeen fifth instars collected from the field were reared to pupation at 16L:8D, while 26 fifth instars were reared to pupation at 8L:16D. Following

pupation, all pupae remained at 15°C, but in darkness, achieved by wrapping pupal storage boxes in aluminium foil.

Trial 2: This trial was designed to test the hypothesis that pupae exposed to a long daylength would develop earlier than pupae held in darkness. Pre-pupal larvae were allocated to one of three pupation environments at 15°C. Six individuals were placed at 16L:8D, five individuals were placed into darkness inside a plastic box wrapped in aluminium foil, while ten individuals were placed in a plastic box, partially filled with soil, in which they spun a cocoon and pupated.

Trial 3: This trial tested the same hypothesis as Trial 2, but had an additional dark treatment and between 34 and 42 pupae in each of the four treatments. Larvae were allowed to develop to the pre-pupal stage in an outdoor insectary before being transferred to 15°C. Prepupae were collected from the insectary every 2-3 days between 17th September and 15th October 1996. Prepupae were allocated to one of four pupation environments, all at 15°C, where they remained until adult eclosion:

i) Naked-Lit: prepupae placed at 16L:8D.

ii) Naked-dark: prepupae placed in darkness

iii) Soil-Lit: prepupae placed in plastic boxes filled with soil.

iv) Soil-Dark: prepupae placed in plastic boxes filled with soil and also wrapped in aluminium foil.

Prepupae transferred to the naked-dark and soil treatments were conservatively estimated to pupate 14 days later, as mean prepupal duration at 15°C ranged from 10.4 ± 0.2 (SE) days (n = 69) to 11.9 ± 0.1 days (n=68) in developmental studies (Chapter 3).

Monitoring development

Pupae held at 11.5°C (16L:8D) and 15°C (16L:8D) were examined every 1-2 weeks under a stereo-microscope for changes in pupal appearance as pupae developed. Pharate adults were checked 2-3 times weekly for adult eclosion. Pupae in the three 'daylength' trials were first examined for visible signs of development (described in Section 2.3.1) 45 days after pupation. Pupae in soil treatments were gently teased out of their cocoons, checked briefly for development, then returned to their cocoons and re-buried in soil. Thereafter, pupal storage boxes in the daylength trials were checked weekly for emerged adults. The time when pupae in 'dark' treatments were exposed to light was kept to a minimum, generally around 5-10 seconds per check.

Eclosion dates were recorded as moths emerged, at which time pupal duration was determined by calculating the number of days that had elapsed since pupation. Once all pupae had completed development, pupal duration at 11.5 and 15°C was examined in relation to the time of year that fifth instars were collected. Linear regressions were used to quantify the relationship between larval collection time and both pupal duration and adult emergence time. Pupal duration at 15°C was then examined separately within each of the above daylength trials to assess the effect of different rearing conditions on pupal duration. Mean pupal durations among the different treatments within each trial were compared using T-tests, as the number of pupae completing development per treatment was always below 30.

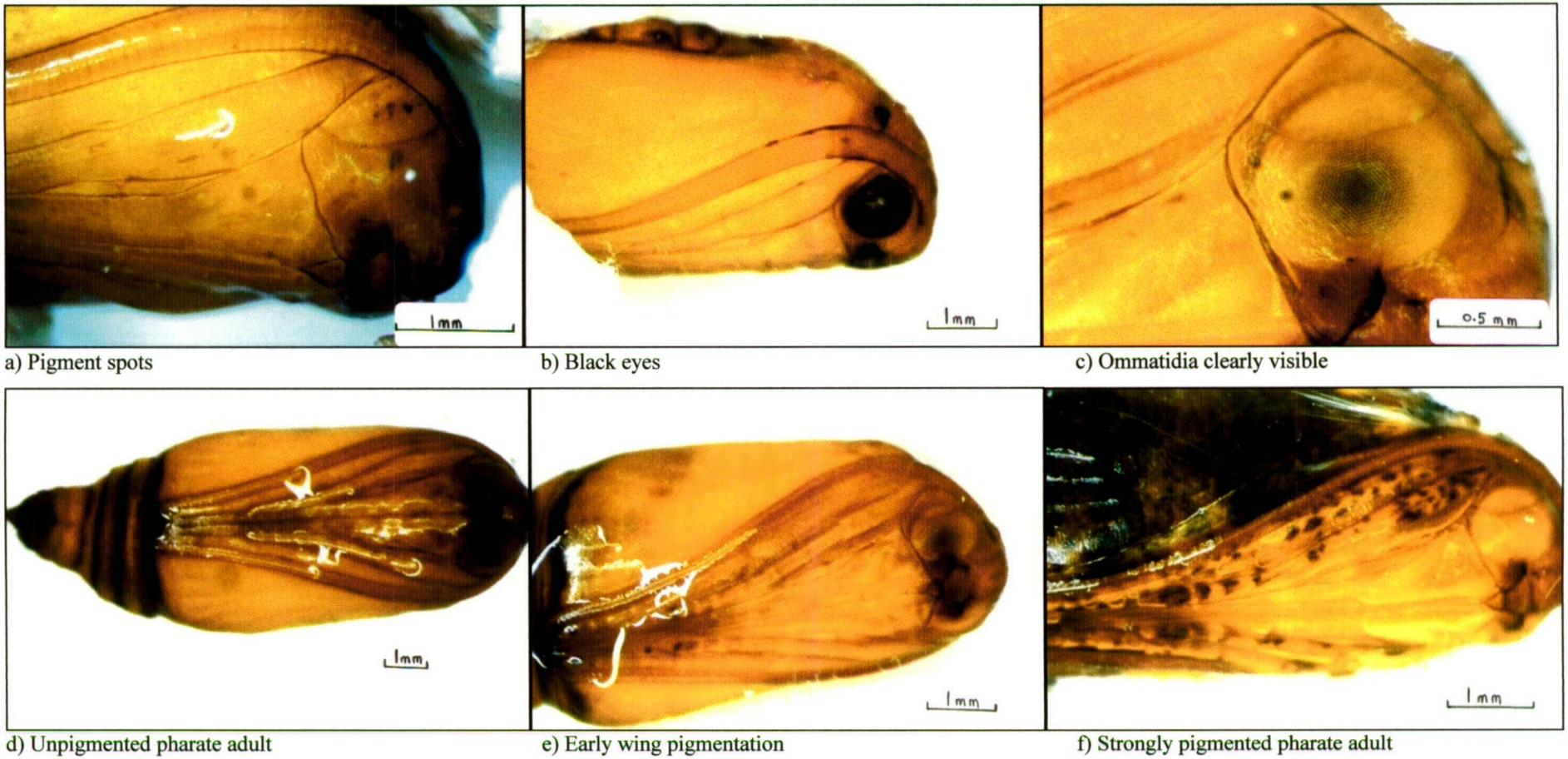
2.3 RESULTS

2.3.1 Pupal development

Examination of pupae under a stereo-microscope revealed distinct changes in pupal appearance during development. Following pupation the head of the pupal stage possesses a characteristic row of three or four black pigment spots in the eye region (Fig. 2.4a). The first visible sign of *M. privata* pupal development is a dorsal shift in

the position of these black pigment spots. This is clearly visible through the transparent, amber-colored pupal cuticle. Several days after pupal development commences, the thoracic region develops a cloudy appearance, presumably the result of cell differentiation. A speckled film over the eye region signals the start of development of the compound eye. Ommatidia soon become clearly visible through the cuticle, after which the eyes develop strong pigmentation and turn black (Fig. 2.4b). The eyes remain black until all major features of the adult moth, such as legs, tarsal hairs and haustellum, are clearly visible through the cuticle. At this stage, the eyes return to an amber colour and ommatidia become clearly visible again (Fig. 2.4c). The moth appears fully developed at this stage, but lacks any pigmentation. Hence, the developing pupa may be considered an unpigmented 'pharate adult' (Fig. 2.4d) as it is enclosed within a pupal cuticle (Gillott, 1995). Pigmentation of the body begins at the wing margins, progresses across the wing pads (Fig. 2.4e) and culminates with pigmentation of the legs and abdomen (Fig. 2.4f). Finally the adult emerges, an event known as eclosion. These developmental stages are easily and rapidly observed under a stereo-microscope and, as the method of examination is non-destructive, pupae may be examined repeatedly as they develop.

The mean number of days that elapsed between the onset of adult differentiation, indicated by a dorsal shift in the position of eye pigment-spots, and adult eclosion was 86.3 ± 1.0 (SE) (n = 46, range = 71-100d) at 11.5°C and 54.3 ± 0.7 (n=58, range = 40-66d) at 15°C. However, as the following results show, the duration of the pupal stage was highly variable and could last up to 4-5 times longer than the mean period required to complete adult differentiation.



a) Pigment spots b) Black eyes c) Ommatidia clearly visible

d) Unpigmented pharate adult e) Early wing pigmentation f) Strongly pigmented pharate adult

Figure 2.4 Visible characteristics of *M. privata* pupae that were used to identify the developmental stage during adult differentiation. a) Pupa with a row of eye spots before adult differentiation begins. b) Pupa at the 'black-eye' phase of development. c) Ommatidia visible through the pupal cuticle. d) Unpigmented pharate adult. e) Pharate adult shortly after the start of wing pigmentation. Legs, haustellum and antennae are visible through the transparent cuticle. f) Strongly pigmented pharate adult just before eclosion.

2.3.2 *Pupal duration of* M. privata

Pilot study (1995)

The mean pupal duration of *M. privata* following each larval collection date during the 1995 season is presented in Table 2.3. Adult moths emerged from 59 of 104 pupae, while parasitoids emerged from a further 8 pupae and the remaining 37 pupae died before moths or parasitoids emerged (Table 2.3). The mean duration of the pupal stage of *M. privata*, based on all eight groups, was 128.3 ± 8.2 (SE) days (n=59) (range 40-288 d) at temperatures between 10 and 22°C (Table 2.3). However, the mean pupal duration within a group ranged from 67.5 ± 7.5 (n=2) to 256.3 ± 18.2 (n=3). Significant negative correlations were found between pupal duration and the time of larval collection, first at 16.5°C ($r = -0.55$, $p<0.01$) (Fig. 2.5a) and then for all groups (except 19.Feb.95) combined ($r = -0.62$, $p<0.01$) (Fig. 2.5b). Excluding the 19.Feb.95 group, predicted pupal duration declined from a mean of 231 days on 5th April 1995 by 0.9 days for each day between 5th April and the larval collection date (Regression: $F_{1,46} = 29.12$; $p<0.001$; $r^2 = 0.39$) (Fig. 2.5b).

The emergence times of *M. privata* adults in relation to larval collection time in 1995 are presented in Fig. 2.6. Two emergence periods can be distinguished: autumn-winter 1995 and spring 1995 to early autumn 1996. The first emergence period, lasting from 10th April to 3rd July 1995, consisted only of adults reared from larvae collected at *Surrey Hills* on 19th February 1995. The second emergence period, lasting from 6 September 1995 to 20 March 1996, consisted of adults reared from larvae collected at both *Surrey Hills* and Hobart between 5th April and 31st October 1995. In contrast to pupal duration, the time of adult emergence was not related to larval collection time between April and October ($r = 0.04$, $p=0.78$), with less than one percent of total variation in emergence time being accounted for by

Table 2.3 The fate of *Mnesampela privata* pupae derived from fifth instars collected in the field during the first year of the study (1995).

Larval Group	Collection Date (1995)	Locality	Pupae n	Moths n	Pupal duration (days) Mean ± SE	Range	Rearing Temp. (°C)	Parasitized [c] n	Died n
1	19-Feb	*Surrey Hills*	26	11	80.8 ± 7.6	40 - 124	16.5, 10 [a]	1	14
2	5-Apr	*Surrey Hills*	5	3	256.3 ± 18.2	225 - 288	16.5, 10, 20 [b]	1	1
3	11-May	*Surrey Hills*	9	6	174.7 ± 17.3	115 - 222	16.5	0	3
4	12-Jul	*Surrey Hills*	12	7	154.3 ± 18.1	52 - 189	22	0	5
5	13-Jul	Hobart	5	5	184.2 ± 35.5	43 - 226	22	0	0
6	2-Aug	*Surrey Hills*	11	9	106.6 ± 15.1	53 - 159	16.5	1	1
7	11-Aug	Hobart	22	16	110.4 ± 11.3	54 - 186	16.5, 10, 20 [b]	0	6
8	31-Oct	*Surrey Hills*	14	2	67.5 ± 7.5	60 - 75	16.5	5	7
Totals			104	59	128.3 ± 8.2	40 - 288	10 - 22	8	37
Proportion			1.00	0.57				0.08	0.35

[a] With the exception of the first moth in cohort 1, which emerged after 40 days at 16.5°C, all moths in cohort 1 emerged at 10°C after spending the first 40 days of the pupal period at 16.5°C and the remaining period at 10°C.

[b] Refers to cohorts in which some moths only emerged at 20°C after initially remaining dormant for long periods at 16.5°C and then at 10°C, to which pupae were transferred in an attempt to break pupal dormancy.

[c] Pupal parasitism rates are presented in Table 2.3 only to show the fate of all pupae. Further details of pupal parasitism are presented in Appendix A, which presents information on the parasitoids of *M. privata* that were encountered during the study.

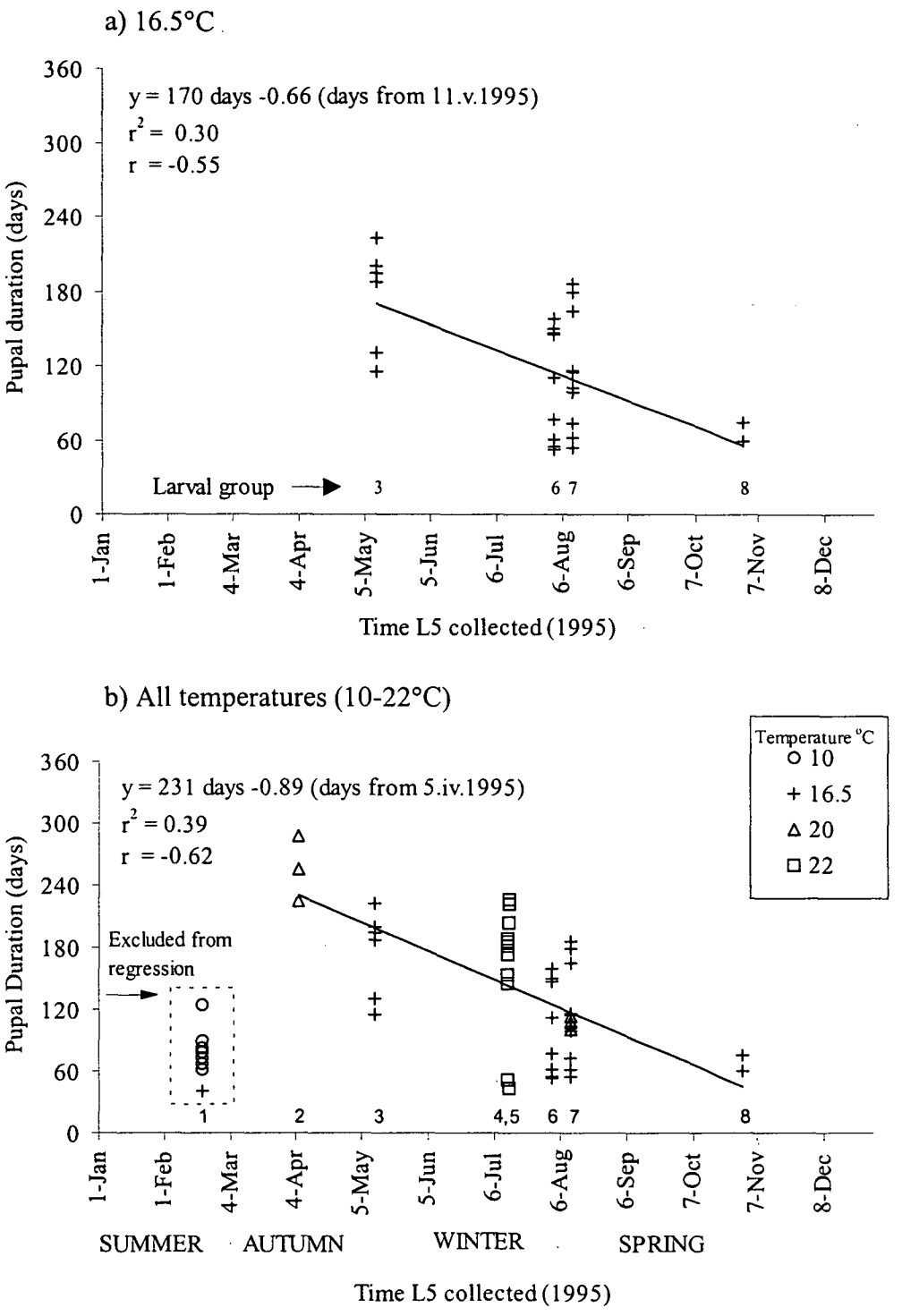


Figure 2.5 The pupal duration of *M. privata* in relation to the time of year that fifth-instars were collected from the field in 1995. The top figure (a) refers to moths that emerged at 16.5°C, while the bottom figure (b) refers to moths that emerged at all temperatures, ranging from 10-22°C.

Notes:

- Dates on x-axis labels change from month to month because the interval between labels is 31 days.
- Moths in group 1 that emerged at 10°C had spent the first 40 days of the pupal stage at 16.5°C.
- Moths in groups 2 and 7 that emerged at 20°C had spent up to six months at 10-16.5°C, where they remained dormant.
- The 22°C temperature for cohorts 4-5 is approximate and refers to moths that developed on a laboratory bench at room temperature.

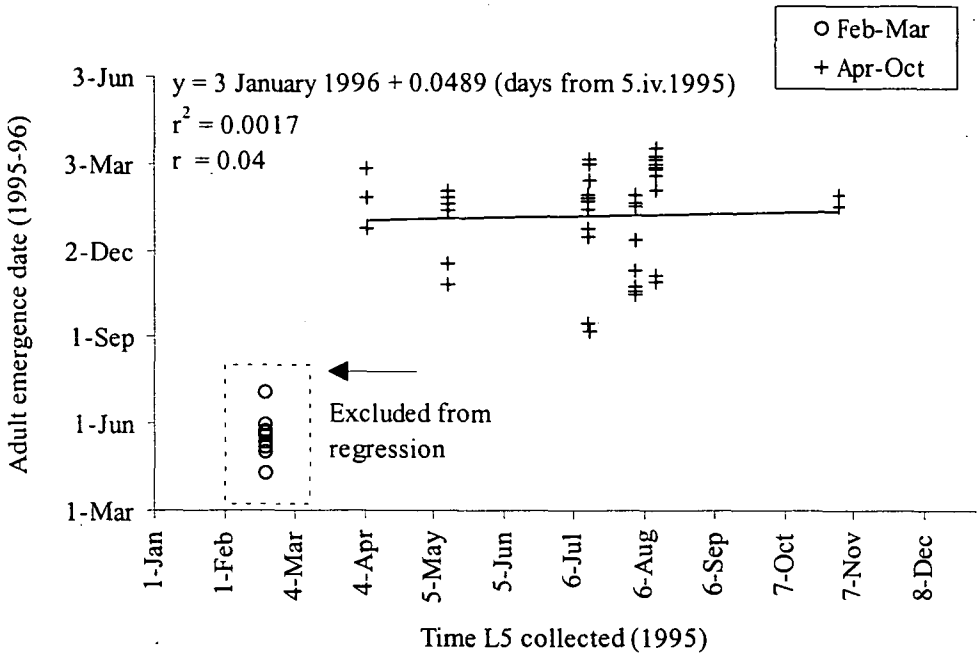


Figure 2.6 The date of *M. privata* adult emergence in the laboratory in relation to the time of year that fifth-instars were collected from the field in 1995. The interval between x-axis labels is 31 days, between y-axis labels is 92 days.

variation in larval collection time (Fig. 2.6). The mean and median emergence dates among the April-October groups were 8th and 22nd January 1996 respectively.

Main Study (1996)

The mean pupal duration of *M. privata* following each larval collection period during the 1996 season is presented in Table 2.4. Adult moths emerged from 347 of 665 pupae, while parasitoids emerged from 63 pupae and the remaining 255 pupae died before moths or parasitoids emerged (Table 2.4). Pupal duration varied markedly at both incubation temperatures, ranging from 100-378 days at 11.5°C and from 49-265 days at 15°C (Table 2.4). Consistent with 1995 results, significant negative correlations were found between pupal duration and the time of year that larvae were collected in 1996 (Fig. 2.7 and Fig. 2.8a). After combining groups 9-15, predicted pupal duration at 11.5°C declined from a mean of 294 days on 20 March by around 0.84 days for each day between 20 March and the larval collection date (Regression: $F_{1,104} = 141.85$, $p < 0.001$; $r^2 = 0.58$) (Fig. 2.7). One individual in group 9 with a

Table 2.4 The fate of *Mnesampela privata* pupae derived from mature larvae collected in the field during the second year of the study (1996).

Rearing Temp. (°C)	Larval Group	Collection Dates (1996)	Locality	Pupae n	Moths n	Pupal duration Mean ± SE	Range	Parasitized n	Died + TR[a] n
15°C	1	15-Feb	*Surrey Hills*	32	6	134.8 ± 23.0	76 - 217	11	10 + 5
	2	22-Feb	Hobart	14	10	82.0 ± 3.4	65 - 98	1	2 + 1
	3	5-8th Mar	Victoria	31	15	96.9 ± 12.5	58 - 242	3	7 + 6
	4	12-March	Hobart	6	2	133.5 ± 15.5	118 -149	2	2
	5	Jun-July	Hobart	43	27	239.2 ± 3.7	182 - 265	0	16
	6	9-August	Hobart	21	17	197.6 ± 2.8	174 - 213	1	3
	7	Sep-Oct[c]	Hobart	148	104	175.5 ± 2.8	49 - 206	6	38
	8	Aug-Nov	Hobart	48	28	160.6 ± 6.0	67 - 227	0	20
Total				343	209	171.8 ± 3.4	49 - 265	24	98 + 12
11.5°C	9	20-March	Tarraleah 1[b]	58	24	325.1 ± 11.5	100 - 378	6	20 + 8
	10	20-March	Tarraleah 2[b]	43	11	306.4 ± 8.1	265 - 355	13	14 + 5
	11	2-April	*Surrey Hills*	41	6	257.3 ± 19.9	207 - 351	4	31
	12	9-May	*Surrey Hills*	51	11	222.6 ± 8.8	167 - 267	9	31
	13	10-May	Tarraleah 1[b]	58	33	230.4 ± 5.8	134 - 282	3	18 + 4
	14	10-May	Tarraleah 2[b]	50	10	223.0 ± 10.9	171 - 286	4	34 + 2
	15	1-Oct	Hobart	21	12	157.8 ± 3.2	133 - 174	0	9
Total				322	107	251.3 ± 6.3	100 - 378	39	157 + 19
Grand Total				665	316	198.8 ± 3.7	49 - 378	63	255 + 31
Proportion				1.00	0.48			0.09	0.38 + 0.05

[a] +TR refers to pupae transferred to temperatures higher than 11.5 and 15°C after the onset of adult differentiation. Pupal periods at temperatures other than 11.5 and 15°C are not included in the mean and range columns above.

[b] Larvae were collected from two plantations in the Tarraleah region on both 20 March and 10 May 1996. Separate results are shown for each plantation.

[c] Group 7 was collected in Hobart on 11th September 1996 and allowed to develop to the pre-pupal stage in an outdoor insectary before being transferred to 15°C. Prepupae were collected from the insectary every 2-3 days between 17th September and 15th October 1996. In that group, the date that prepupae were transferred from the insectary to the 15°C incubator was used as the collection date during data analysis.

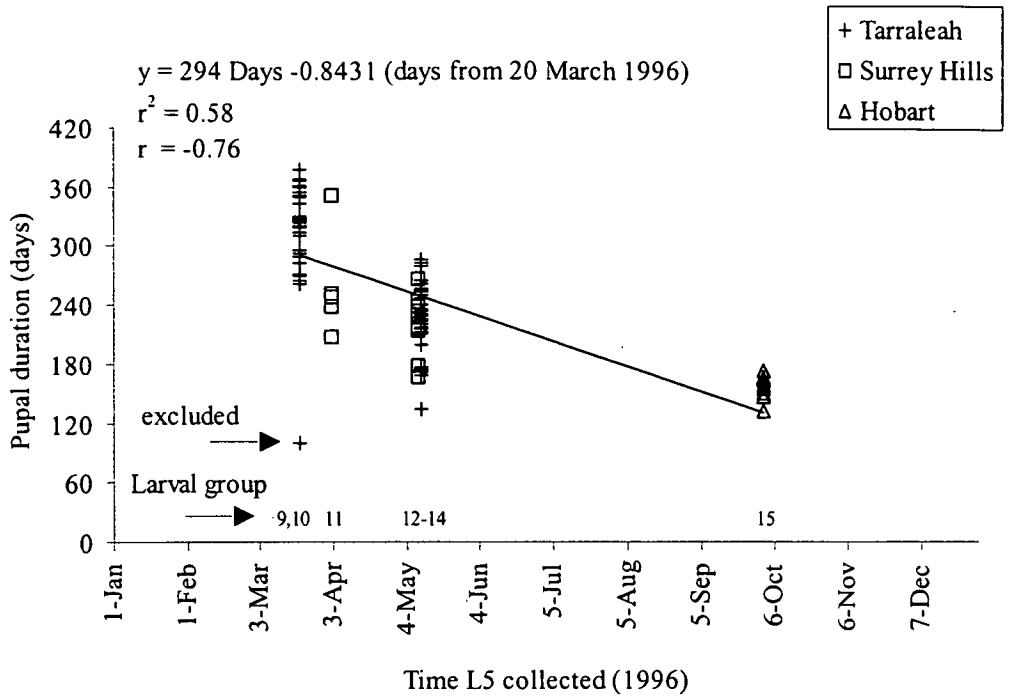


Figure 2.7 The pupal duration of *M. privata* at 11.5°C following collection of fifth-instars between 20 March and 1 October 1996. The x-axis interval is 31 days.

pupal duration of 100 days was excluded from the regression as it was hypothesised to represent a single pupa with non-diapause development. Reasons for this diapause hypothesis are given in the discussion.

At 15°C, individuals collected before mid-March tended to have a relatively short pupal period, as 26 moths emerged within 58-118 days of pupation compared with 7 moths that emerged after a pupal period of 147-242 days (Fig. 2.8a). Pupae from Tasmania and Victoria exhibited this same pattern (Fig 2.8a). Thereafter, after combining groups 5-8, pupal duration was again significantly negatively correlated with the time of year that larvae were collected ($r = -0.86$, $p<0.01$). Predicted pupal duration among groups 5-8 declined from a mean of 252 days on 1 June 1996 by around 0.67 days for each day between 1 June 1996 and the larval collection date (Regression: $F_{1,169} = 488.95$; $p<0.001$; $r^2 = 0.74$) (Fig. 2.8a). Five individuals with pupal durations ranging from 49-68 days were excluded from the regression as they were also hypothesised to represent non-diapause pupal development. When all

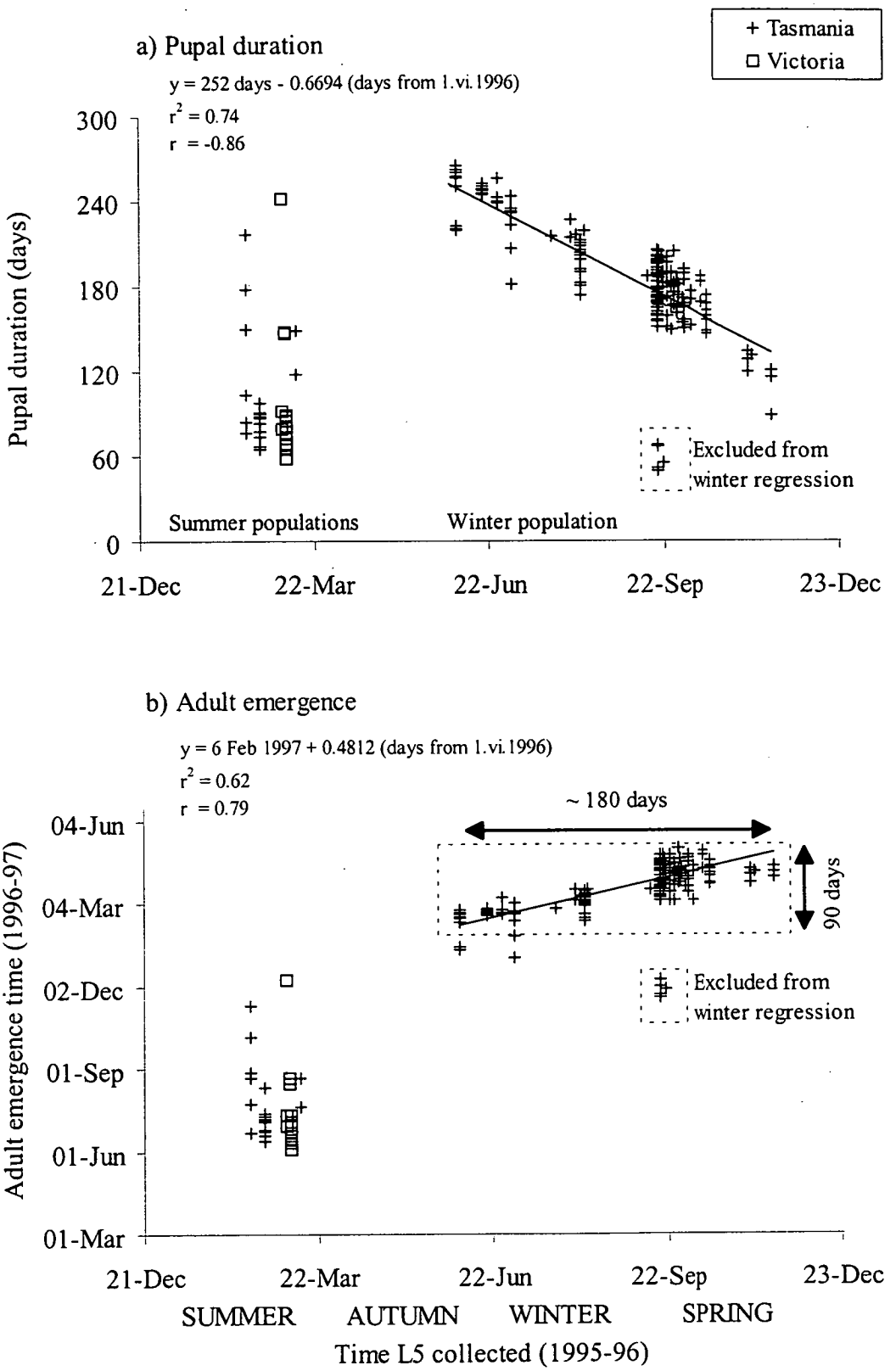


Figure 2.8 The pupal duration (a) and time of adult emergence (b) of *M. privata* at 15°C in relation to the time of year that fifth instars were collected from the field in Tasmania and Victoria in 1996. Excluded individuals were hypothesised to have different diapause characteristics to the majority of individuals collected during winter.

groups collected after mid-March 1996 (i.e. groups 5-15) were combined (ignoring incubation temperature), 71.1% of the variation in pupal duration was accounted for by variation in larval collection time. Pupal duration among these groups declined steadily from a predicted mean of 291 days on 20th March 1996 by around 0.62 days for each day between 20 March and the final larval collection date of 18th November 1996 (Regression: $F_{1,275} = 676.64$; $p < 0.001$; $r^2 = 0.71$) (Fig.2.9).

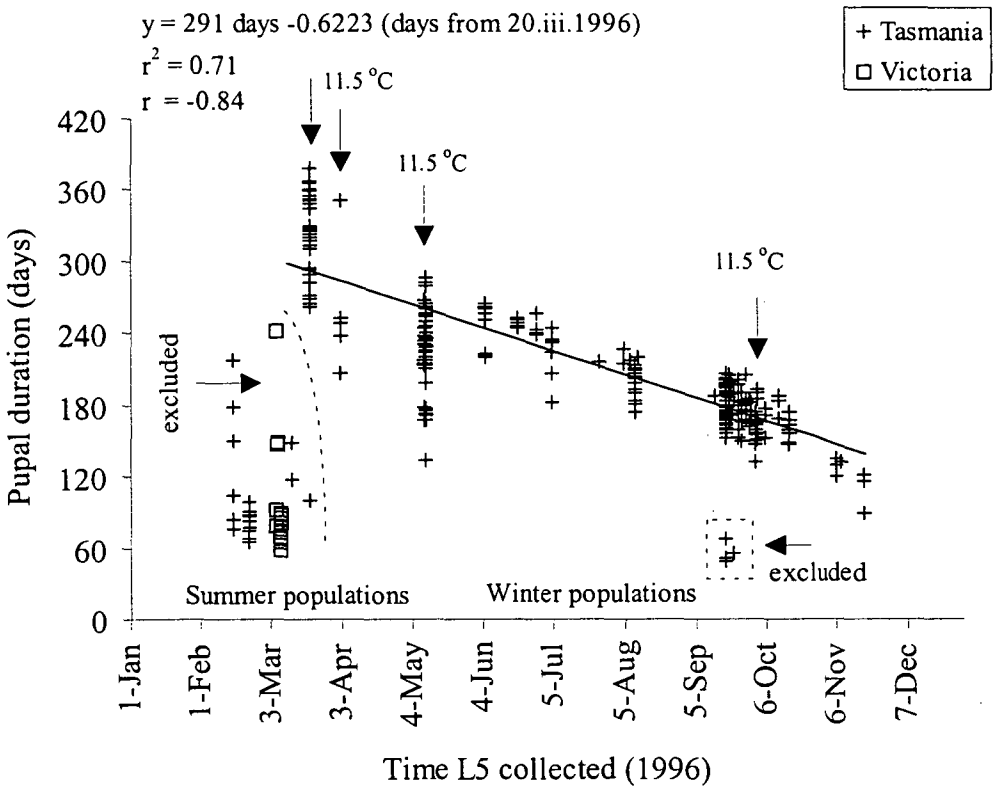


Figure 2.9 The pupal duration of *M. privata* at 11.5-15°C in relation to the time of year that larvae were collected between 15 February and 18 November 1996. Vertical arrows indicate pupal groups that developed at 11.5°C. The vertical arrow above the September-October data points refers only to 12 individuals in group 15, collected on 1 October, that were reared at 11.5°C. Those individuals emerged in synchrony with others at 15°C. Horizontal arrows indicate pupae excluded from the regression analysis used to quantify the seasonal decline in pupal duration between 20 March and 18 November. Excluded pupae were hypothesised to have different diapause characteristics to the majority of individuals.

The emergence times of *M. privata* adults at 15°C in relation to collection time in 1996 are presented in Fig. 2.8b. Larvae collected before mid-March usually produced adults before the end of winter, whereas larvae collected in winter and

spring usually produced adults from February to April of the following year (Fig. 2.8b). In contrast to the pilot study, a significant linear relationship was found between adult emergence time and larval collection time during winter and spring in 1996. The predicted emergence date for an individual collected on 1st June 1996 was 6th February 1997, after which the mean emergence date was delayed by approximately 0.5 days for each day that passed between 1 June and the larval collection date (Regression: $F_{1,169} = 274.40$; $p<0.001$; $r^2 = 0.62$) (Fig. 2.8b). This meant that most adults emerged over a three month period (February-April 1997) (Figs. 2.8b and 2.10) despite a six month larval collection period (June-November 1996). In comparison, most adults emerged over a four month period (January-April 1997) at 11.5°C after larval collection over a seven month period (20 March-1 October 1996) (Fig. 2.10).

2.3.3 The influence of post-collection rearing conditions on pupal duration

The effects of post-collection rearing conditions at 15°C on pupal development are presented in Table 2.5. In Trial 1, the mean pupal duration of 222.1 ± 7.8 d (n=8) for individuals reared to pupation at 16L:8D was significantly shorter than 246.4 ± 2.8 d (n=19) for individuals reared to pupation at 8L:16D ($p<0.01$). However, no adults emerged within 40-60 days in either group. The range in pupal duration in Trial 1 was 182-265 days (Table 2.5). In Trial 2, the mean pupal duration among cocooned pupae in the soil (205.0 ± 2.4 d; n=8) was significantly longer than the mean pupal durations among illuminated (190.2 ± 4.8 d; n=5) and dark (192.0 ± 6.3 d; n=4) pupae held 'naked' in vial lids ($p<0.05$ in both cases), but mean pupal durations among the two naked treatments were not significantly different ($p=0.82$) (Table 2.5). However, as in Trial 1, no adults emerged within 40-60 days of pupation in any of the three treatments, ranging instead from 174-213 days. Finally, in Trial 3 the mean pupal durations among the two soil treatments and the naked dark treatment

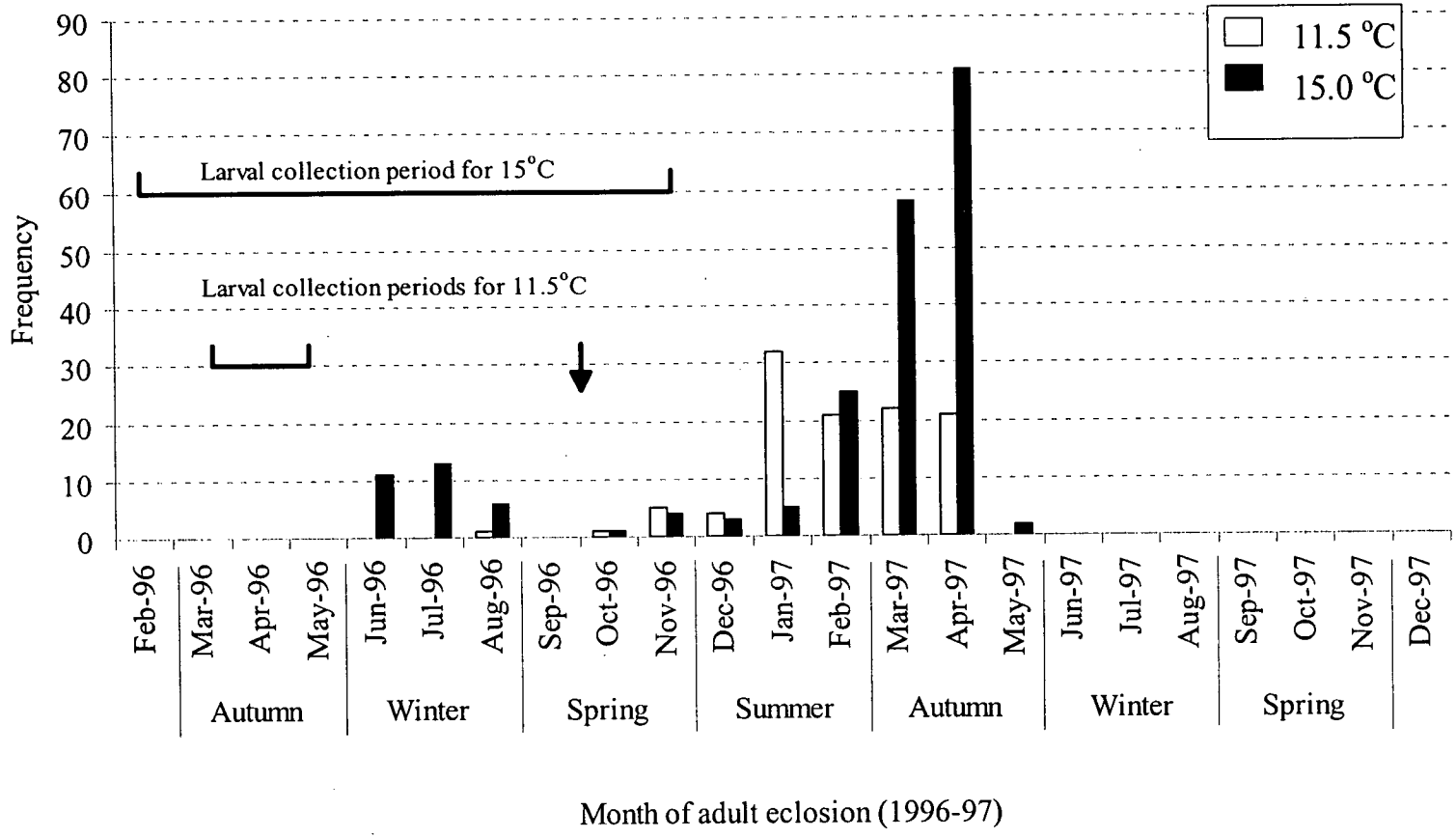


Figure 2.10 The distribution of adult eclosion times of *M. privata* at two incubation temperatures. Individuals at 15°C were collected from the field as fifth instars between February and November 1996, while individuals at 11.5°C were collected from the field as fifth instars from March to May and again on 1st October 1996 (arrow).

Table 2.5 Fate of pupae reared at 15°C in relation to different lighting conditions. Fifth instars were collected from the field during winter and spring in 1996.

Trial No.	Collection date (1996)	Stage Affected	Lighting conditions at 15°C	No. Pupae	No. alive after 45 days	Ratio of Developing: Dormant pupae at 45 days	Number of adults emerging: Early : Late	Pupal Duration (Mean ± SE) Early	Pupal Duration (Mean ± SE) Late	Range (Days) Early	Range (Days) Late
1	5.vi - 25.vii	L5 - pupa	16L:8D	15	15	0 : 15 [+]	0 : 8	n/a	222.1 ± 7.8 a[Ψ]	n/a	182 - 251
			8L:16D	25	24	0 : 24 [+]	0 : 19	n/a	246.4 ± 2.8 b**	n/a	216 - 265
2	9.viii	Pre-pupa to adult	16L:8D	6	6	0 : 6	0 : 5	n/a	190.2 ± 4.8 a	n/a	181 - 202
			0L:24D	5	4	0 : 4	0 : 4	n/a	192.0 ± 6.3 a	n/a	174 - 202
			Soil	10	10	0 : 10	0 : 8	n/a	205.0 ± 2.4 b*	n/a	191 - 213
3	17.ix - 15.x	Pre-pupa to adult	16L:8D Naked-Lit	42	36	5 : 31	4 : 25	55.8 ± 4.3	168.0 ± 2.1 a λ	49 - 68	147 - 191
			0L:24D Naked-Dark	37	34	0 : 34	0 : 25	n/a	186.8 ± 2.5 b**	n/a	160 - 206
			Soil - lit	34	34	0 : 34	0 : 26	n/a	183.7 ± 2.6 b**	n/a	152 - 200
			Soil - dark	34	34	0 : 34	0 : 24	n/a	182.5 ± 3.4 b**	n/a	150 - 202
Total				208	197	5 : 192	4 : 144	55.8 ± 4.3	193.4 ± 2.3	49 - 68	147 - 265

[+] Pupae in Trial 1 were transferred to darkness the day after pupation and, except for brief checking periods lasting 5-10 seconds, remained in darkness until adult eclosion.

[Ψ] Within each trial (i.e. between lines), means followed by the same letter are not significantly different. Probabilities are based on T-tests: * $p<0.05$, ** $p<0.01$.

λ The mean pupal duration of the Naked-Lit treatment in Trial 3 declines to 152.5 ± 7.5 when the four early emerging individuals are included. This overall mean is also significantly different from the other three treatments in Trial 3 at the $p<0.01$ level.

Table 2.6 Summary of the three daylength trials used to test the general hypothesis that exposure to a long daylength during the fifth instar or the pupal stage may cause early adult eclosion in the laboratory.

Trial	Hypothesis	Treatment	Summary Outcome	Possible explanation
1	Exposure to a long daylength during the fifth instar averts pupal diapause and causes adult eclosion within 40-60 days of pupation.	Fifth instars reared to pupation at 16L:8D and 8L:16D. Pupae then reared to adult eclosion in darkness.	No adults emerged in 40-60 days. All individuals had long pupal periods, ranging from 182-265 days.	Pupal diapause was induced prior to larval collection and was not averted by exposing fifth instars to long daylengths.
2	Exposure to a long daylength during the pupal stage averts pupal diapause and causes adult eclosion within 40-60 days of pupation.	Prepupae placed at pupation environments of 16L:8D (Naked-Lit), 0L:24D (Naked-Dark) and into a container of soil (Soil treatment).	No adults emerged in 40-60 days. All individuals had long pupal periods, ranging from 174-213 days.	Pupal diapause was induced prior to larval collection and was not averted by exposing pupae to long daylengths.
3	As for Trial 2.	Prepupae placed at pupation environments of 16L:8D (Naked-Lit), 0L:24D (Naked-Dark), a container of soil (Soil-Lit) and a container of soil wrapped in foil (Soil-Dark).	Four out of 29 adults (14%) from the Naked-Lit treatment emerged within 49-68 days of pupation. Remaining individuals from Naked-Lit and other three treatments had long pupal periods, ranging from 147-206 days.	A small proportion (14%) of Naked-Lit pupae developed without a diapause. All other pupae diapaused. Thus, diapause may have been induced in the majority of individuals prior to larval collection and was probably not averted by long days.

were not significantly different from each other ($p>0.05$), ranging from 182.5 ± 3.4 d (n=24) to 186.8 ± 2.5 d (n=25), but they were all significantly longer than the mean pupal duration 152.5 ± 7.5 d (n=29) in the naked-lit treatment (Table 2.5). The latter mean was biased by four individuals with pupal periods ranging from 49-68 days, but even after excluding those individuals the mean pupal duration of 168.0 ± 2.1 d (n=25) was still significantly shorter than the other three treatments, indicating that pupae exposed to a 16 hr photoperiod produced adults about two weeks earlier than pupae reared in darkness. However, the fact that only 4 of the 29 pupae exposed to a 16 hr daylength in Trial 3 produced adults within 49-68 days shows that holding pupae under long daylengths did not consistently cause adult eclosion within 40-60 days as was originally hypothesised. Thus, there was very little evidence from the three daylength trials to support the hypothesis that exposure to a 16 hr daylength during the fifth instar or the pupal stage caused adult eclosion within 40-60 days of pupation (Table 2.6).

2.4 DISCUSSION

2.4.1 Variability in pupal duration

The mean duration of the pupal stage for eight groups of pupae kept over the 1995-96 period in this study was 128.3 ± 8.7 (SE) days (n=59) (Table 2.3) and for 14 groups kept over the 1996-97 period it was 198.8 ± 3.7 d (n=316) (Table 2.4). The mean pupal duration of 187.7 ± 8.9 days reported by Elliott and Bashford (1978) falls between the two mean values of this study. Elliott & Bashford (1978) noted that most larvae pupated in September and October in southern Tasmania. Reference to Figure 2.9 shows that most individuals collected in September-October 1996 in this study also had a pupal duration of around six months. Hence, the six-month pupal period reported previously is consistent with the findings of this study but reflects only a part of the spectrum of pupal durations found over an entire season (Fig. 2.9).

The duration of the pupal stage of *M. privata* was highly variable, ranging from 40-288 days in 1995 and 49-378 days in 1996. The emergence of some adults within 40-60 days of pupation in the pilot study was highly unexpected, given that a six-month pupal duration was originally anticipated from Elliott & Bashford (1978). When adults first emerged after such a short pupal period, it was hypothesised to be an aberration caused by the laboratory rearing conditions: e.g. perhaps short pupal periods were the result of higher temperatures than those used by Elliott & Bashford (1978). Because of the discrepancy with Elliott and Bashford's (1978) study, discussions were held with R. Bashford (Forestry Tasmania, Hobart) to determine the pupal rearing conditions used in the original study. Bashford (pers. comm. 8th May 1995) stated that pupae had been reared on a laboratory bench at approximately 16-20°C. Therefore, since the six-month pupal period previously reported had occurred at similar temperatures to those used in the pilot study, high incubation temperatures were unlikely to be responsible for the early emergence of some adults. The early emergence could also not be explained by population differences because short and long pupal periods were exhibited in groups collected from both *Surrey Hills* and Hobart (compare groups 6 and 7 in Table 2.3 and Fig. 2.5).

The variability in the pupal duration of *M. privata* strongly resembled the pattern of variability in the pupal duration of *Helicoverpa punctigera* reported by Cullen & Browning (1978). The pupal duration of *H. punctigera* at 28°C ranged from 10-228 days (Cullen & Browning, 1978). In comparison, the pupal duration of *M. privata* ranged from 100-378 days at 11.5°C, from 49-265 days at 15°C, from 40-222 days at 16.5°C, and from 43-226 days at 22°C. Variability in the pupal duration of *H. punctigera* was mainly due to the presence or absence of a pupal diapause that could last up to six months (Cullen & Browning, 1978). Therefore,

one possible explanation for variability in the pupal duration of *M. privata* could be that pupae of *M. privata* have the capacity to enter a diapause.

For the following discussion, it is necessary to briefly re-visit the definitions of the terms diapause and quiescence. Diapause is induced by token stimuli in the environment, e.g. daylength, which, although not detrimental themselves, reliably predict the approach of unfavorable conditions, whereas quiescence occurs in direct response to adverse conditions (Beck, 1968; Tauber *et al.*, 1984; Denlinger, 1985). Because diapause anticipates the arrival of adverse conditions, further development is prevented even under favorable conditions. Hence, when diapause and non-diapause individuals are held under identical conditions that are favourable for development, diapause individuals may remain dormant for long periods while non-diapause individuals complete development rapidly. The fact that pupae developed at temperatures between 10°C and 22°C in the pilot study and at 11.5 and 15°C in the main study indicates that temperatures between 10 and 22°C are favourable for pupal development. Therefore, non-development at these temperatures is unlikely to be due to quiescence, and is thus likely to be due to a pupal diapause. Cullen and Browning (1978) considered a pupal duration exceeding 20 days at 28°C to be an indication of pupal diapause in *H. punctigera*. Since *M. privata* pupae can complete adult differentiation in around 80-90 days at 11.5°C and 40-60 days at 15-22°C, perhaps a pupal duration exceeding 90 days at 11.5°C and 60 days at 15-22°C could be an indication of pupal diapause in *M. privata*. Extrapolating from these results shows that diapause duration, calculated by subtracting the period of adult differentiation from total pupal duration, lasted up to 288 days at 11.5°C and up to 162-205 days at 15-22°C.

The diapause hypothesis is further supported by a strong resemblance between non-developing *M. privata* pupae and diapausing *H. punctigera* pupae. Both species have distinct pigment spots in the eye region of the pupal head before pupal development begins (compare Fig. 2.4a with Fig. 1 of Cullen & Browning, 1978) and pupae of both species may remain in this stage for many months at temperatures which are otherwise favourable for pupal development. Furthermore, the earliest visible signs of adult differentiation, i.e. a shift in the position of eye-spots followed by a black-eye phase, are identical in *M. privata* and *H. punctigera*. Eye pigmentation is also used to indicate the onset of pupal development in the cabbage moth, *Mamestra brassicae* (e.g. Grüner & Masaki, 1994). Hence, the assumption that the presence of eye-spots is an indication of pupal diapause in *M. privata* is probably justified as this method is consistent with methods reported in previous studies of the pupal stage of other lepidopteran species.

2.4.2 Seasonal variation in pupal duration

Although pupal duration of *M. privata* was highly variable, the variation was far from being haphazard, tending instead to follow a consistent seasonal pattern. If larval development was completed before mid-March, pupal development was usually completed within 2-4 months of pupation, although longer pupal periods were still possible. For example, the mean duration of the pupal stage was 80.8 ± 7.6 d (n=11) in the 19.Feb.95 group, 82.0 ± 3.4 d (n=10) in the 22.Feb.96 group, and 96.9 ± 12.5 d (n=15) in the 5-8.Mar.96 group. Rapid completion of pupal development early in the season meant that next generation adults could emerge during autumn and winter. This result is extremely important because it demonstrates that *M. privata* is a potentially bivoltine species. Eggs and early instars were found at *Surrey Hills* during April and May 1995 in the same plantations where mature larvae were collected in February (pers. obs.). These eggs and early instars

were probably the source of the mature larvae collected late in the season at *Surrey Hills*. These field observations support the hypothesis that *M. privata* is potentially bivoltine at high altitudes in Tasmania, providing that pupation occurs early in the season. However, given that the pupal stage lasted up to 378 days (Table 2.4), those same field observations may instead reflect oviposition by a sub-population of adults emerging late in the season (for *Surrey Hills*) from an 'old stock' of pupae remaining from the previous year.

That *M. privata* is potentially bivoltine is considered a real possibility. Assuming that pupal periods of 40-60 days at 15°C are an indication of non-diapause pupae and that longer pupal periods are an indication of diapause pupae, then the seasonal incidence of diapause found in *M. privata* resembles the pattern in the cabbage butterfly *Pieris napi* L. (Lepidoptera: Pieridae) described by Danilevskii (1961). In *P. napi*, as in *M. privata*, early-season larvae produce both non-diapause and diapause pupae, meaning that at least some second generation adults emerge in the same season (see Fig. 51 of Danilevskii, 1961). Late-season larvae of both species usually produce diapause pupae, meaning that next generation adults from late-season pupae usually emerge the following season. Thus, the potential for bivoltine behaviour by *M. privata* should not be overlooked at high altitude sites, especially during warm summers when pupation would occur earlier in the season.

A critical point appeared to be reached in the middle of March, at which time pupal duration of *M. privata* increased sharply and reached a seasonal maximum of around 8-10 months. This is illustrated by mean pupal periods of 256.3 ± 18.2 d (n=3) in the 5.Apr.95 group (Table 2.3) and 325.1 ± 11.5 d (n=24) and 306.4 ± 8.1 d (n=11) in the two 20.Mar.96 groups (Table 2.4). Thereafter, pupal duration steadily declined as the season progressed, eventually reaching 4-5 months by the end of the

season, i.e. November (Fig. 2.9). Given that daylength is a reliable indicator of seasonal change and is a widely used token stimulus for diapause induction in insects (Beck, 1968; Denlinger, 1985), daylength may be used as the basis for developmental decisions in *M. privata*. From the results, it is hypothesised that the autumnal equinox (which occurs on 20 or 21 March each year) may be a critical time of year, perhaps a 'switching time' (see Taylor, 1986), separating alternative developmental pathways which may be taken by *M. privata* after pupation (Fig. 2.11). Long daylengths before the autumnal equinox may enable pupal diapause to be averted, while short daylengths after the autumnal equinox may ensure that the majority of pupae enter diapause. This hypothesis is supported by the data collected over two years. Larvae collected before the autumnal equinox usually produced adults in the same year that larvae completed development, whereas larvae collected after the equinox usually produced adults the year after larvae completed development (Figs. 2.6 and 2.8b).

The seasonal decline in pupal duration between April and November meant that although most larvae were collected over a 6-7 month period, adults emerged over a three month period (Fig. 2.8b). Thus, flexibility in pupal duration contributed to synchronised adult emergence the following season. Furthermore, the period of peak adult emergence in the laboratory in 1997 was from January-April (Fig. 2.10) which corresponds to the summer and autumn adult flight periods exhibited naturally by *M. privata* in Tasmania (Elliott & Bashford, 1978) and Figure 2.12.

The decline in pupal duration as the season progressed reflects flexibility in diapause length, as the period of adult differentiation was relatively uniform, at around 54 days at 15°C and 88 days at 11.5°C. Such a seasonal decline in diapause duration is not uncommon, as Danks (1994a) notes that many species that enter

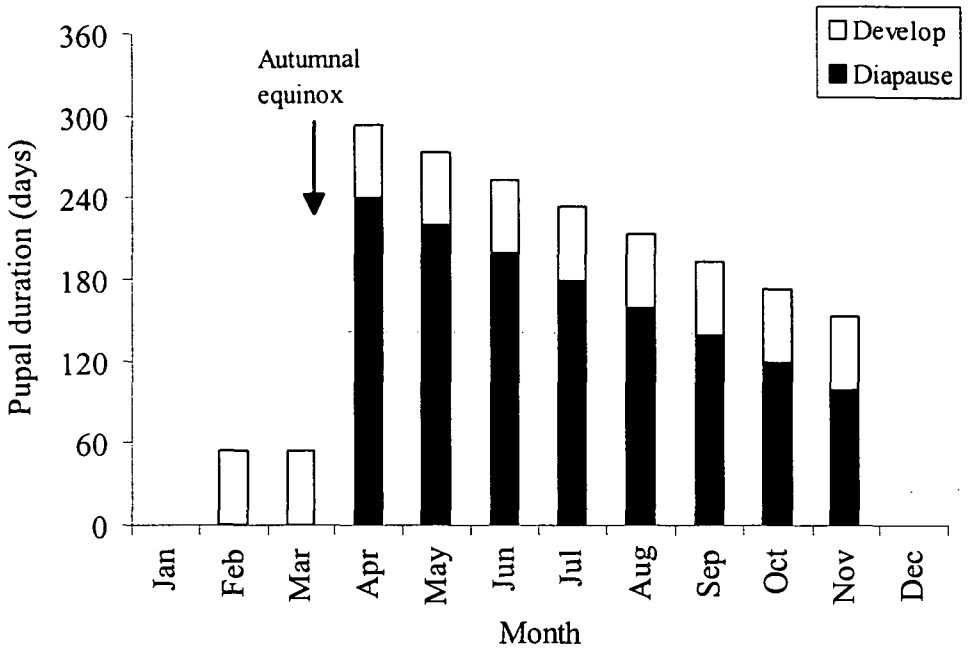


Figure 2.11 Schematic diagram of how induction and duration of pupal diapause in *M. privata* may vary as the season progresses. It is assumed that the autumnal equinox represents a 'switching time' (see Taylor, 1986) separating alternative developmental pathways that may be taken by *M. privata* after pupation. Long daylengths experienced by larvae before the autumnal equinox do not induce pupal diapause. Short daylengths experienced by larvae after the equinox induce pupal diapause in the majority of pupae. Compare with Fig. 2.9.

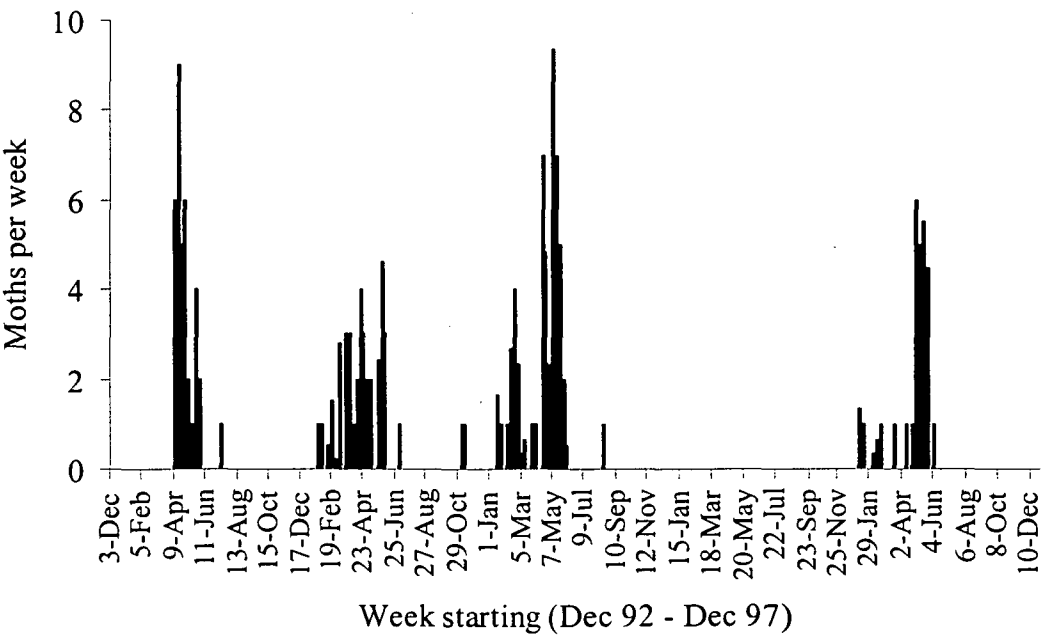


Figure 2.12 Weekly catches of *M. privata* in a 160 watt mercury vapour light trap at Devonport (46 m ASL) in northern Tasmania. No data were available for 1996. Data kindly supplied by L. Hill, Dept. of Primary Industry and Fisheries, Tasmania.

diapause later in the season have a shorter diapause, which contributes to synchronised emergence the following season.

2.4.3 Implications for M. privata *phenology*

I conclude this chapter by introducing a flow chart summarising the existing state of knowledge of factors regulating the phenology of *M. privata* (Fig. 2.13). The use of such flow charts is recommended by Danks (1994a) to help visualise the way that various factors interact to regulate insect life cycles. The flow chart starts with contrasting adult phenologies (summer and autumn), then shows how seasonal variation in the time of larval development and pupation interacts with the autumnal equinox to influence the induction and duration of pupal diapause. Diapause induction and duration in turn are the key factors influencing pupal duration and hence, the phenology of adults that will give rise to the next generation. Bivoltinism appears possible at high altitudes in Tasmania but only if pupation occurs before the autumnal equinox. Pupation after the autumnal equinox usually leads to a pupal diapause of variable duration, which maintains univoltinism and contributes to synchronised adult emergence the following autumn. It must be noted that although a pupal diapause is suspected, it has yet to be proven by further experimentation. The possibility of a pupal diapause in *M. privata* and the underlying mechanisms responsible for diapause induction are further investigated in Chapter 4.

Finally, it must be stressed that the above flow chart is incomplete. The flow chart comes full circle for univoltine populations initiating seasonal activity in autumn but the cause of summer adult phenology has yet to be determined. Hence additional factors need to be identified before the summer phenology of adult *M. privata* at high altitudes in Tasmania can be explained. Further steps will be added to the flow chart in subsequent chapters as new information becomes

available. The following chapter begins the investigation of other factors controlling the phenology of *M. privata* by examining the temperature requirements for the development of each immature stage in the life-cycle.

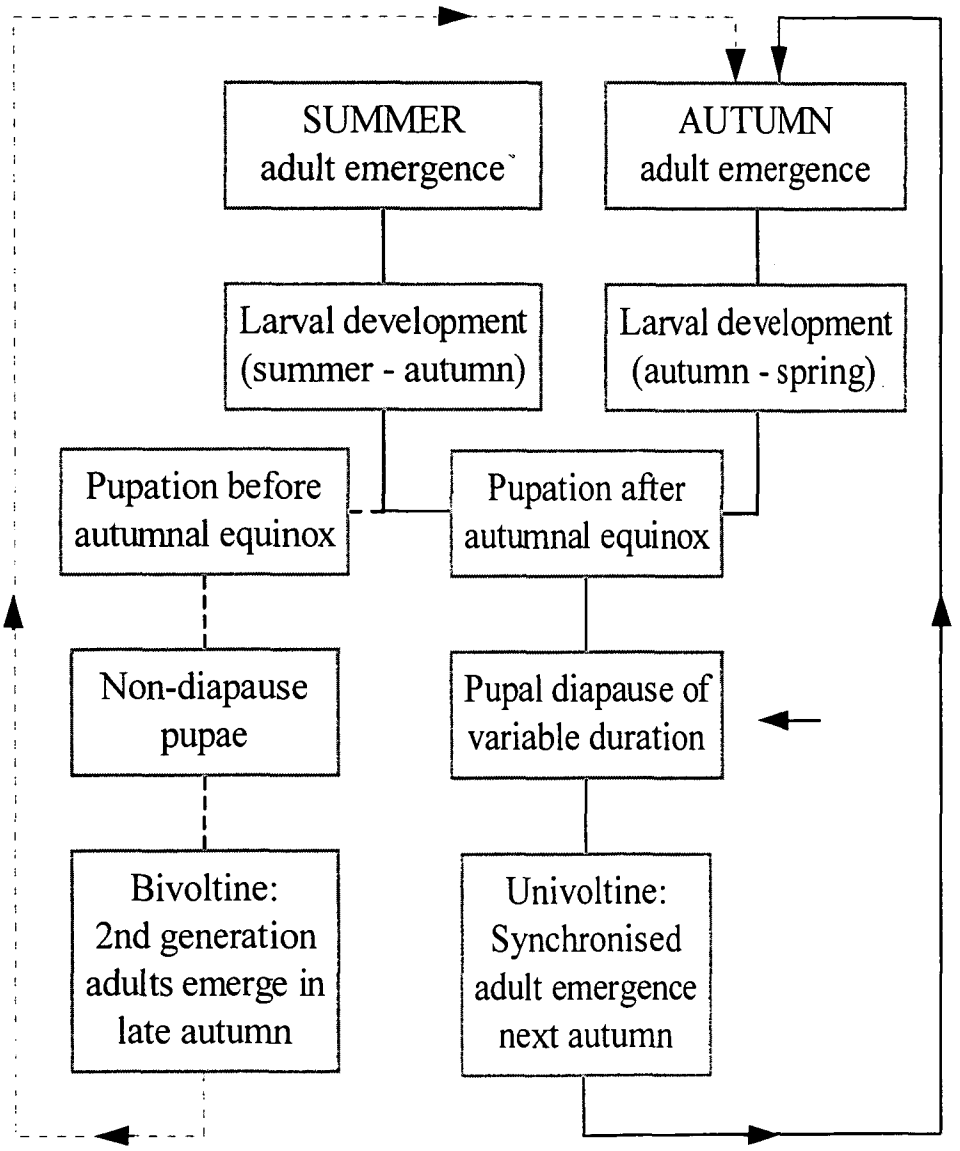


Figure 2.13 Proposed flow chart of factors influencing the phenology of *M. privata*. Adult emergence usually occurs in summer at altitudes above 500 m ASL in Tasmania and in autumn at lower altitudes in Tasmania and throughout mainland Australia. Pupation before the autumnal equinox at high altitudes in Tasmania could give rise to second generation adults in autumn from non-diapause pupae. Pupation after the autumnal equinox leads to pupal diapause and a univoltine life-cycle.

3. GENERATION TIME OF *MNESAMPELA PRIVATA*

3.1 INTRODUCTION

Temperature is the dominant environmental factor affecting the growth and development of insects and other arthropods (Howe, 1967; Campbell *et al.*, 1974; Logan *et al.*, 1976; Gordon, 1984; Gilbert, 1984, 1988). Hence, phenology models relating insect development to temperature are integral parts of management strategies for insect pests because they provide an estimate of the seasonal occurrence of insect pest stages and help guide sampling and control decisions (Tauber & Tauber, 1978; Tauber *et al.*, 1984; Zeiss *et al.*, 1996).

Information on the development times of *M. privata* eggs, larvae and pupae prior to this study was cursory. In the field, eggs took 14 days to hatch during autumn in southern Tasmania and the larval stages lasted two to three months during autumn and winter; while in the laboratory, larval and pupal stages lasted 71.8 ± 3.1 and 187.7 ± 8.9 days (mean ± SE) respectively (Elliott & Bashford, 1978). In north-western Tasmania, eggs and the first two larval instars took 16, 7 and 9 days respectively to develop during mid-summer (de Little, 1981). The reported field durations of the egg and larval stages provide a useful guide to approximate development times in Tasmania once the life-cycle is underway. However, these development times were determined under ambient conditions and the developmental threshold, essential for estimating physiological development time, has never been determined. Hence, development times of *M. privata* needed to be better related to temperature and a developmental threshold determined so that local average temperatures may be used to forecast the insect's local phenology.

The relationship between insect development rate and temperature is sigmoidal with a linear component at intermediate temperatures which correspond to

temperatures normally experienced in the field (Howe, 1967, Campbell *et al.*, 1974; Gilbert, 1988). Because of the linearity of the developmental response at intermediate temperatures, linear regression is commonly used to estimate the relationship between insect development rate and temperature, but only in the linear range (Gilbert, 1988). At temperatures below the mid-range, development rates are faster than predicted, while at temperatures above the mid-range development rates are slower than predicted (Howe, 1967; Gordon, 1984). Non-linear models (e.g. Logan *et al.*, 1976; Berry *et al.*, 1977; Dallwitz & Higgins, 1992) may overcome some of these difficulties but, as noted by Campbell *et al.* (1974), 'the deleterious effect of high temperature is not normally experienced', and field temperatures are usually within or below the linear zone. Thus, although non-linearity of the temperature response at temperatures near the upper and lower developmental thresholds may affect predictions of insect development rates, linear models are used most commonly: e.g. for Homoptera (Campbell *et al.*, 1974), Lepidoptera (Gilbert, 1984, 1988, Allsopp *et al.*, 1990), Diptera (Read & Moon, 1996), Coleoptera (Zeiss *et al.*, 1996) and Hymenoptera (Miller, 1996; Olaye *et al.*, 1997).

The regression line of linear models is best defined by its threshold temperature and its slope (Campbell *et al.*, 1974; Gilbert, 1988). From a typical regression equation $y = a + bx$, where y refers to development rate (1 / days), a is the y intercept, (the value of y when $x = 0$), b is the slope of the regression line, and x is the temperature, the lower developmental threshold (T_0) is approximately the temperature at which the regression line intercepts the x axis (the value of x when $y = 0$) and is estimated as $T_0 = -a / b$. The reciprocal of the slope ($1 / b$) is used to estimate the thermal constant (K) in units of degree-days (DD) above T_0 required to complete development between two recognisable stages, such as between oviposition

and egg hatch or between two successive larval moults (Howe, 1967; Campbell *et al.*, 1974; Gordon, 1984). Standard errors of T_0 and K may also be calculated (Campbell *et al.*, 1974).

Although linear regression is commonly used to quantify the temperature response, an alternative method of estimating T_0 is sometimes possible. Gilbert (1988) showed that the developmental thresholds of 36 warm-season insect species were roughly equal to the average field temperatures at the time of first emergence from winter dormancy. Using this method, the lower developmental thresholds of 'warm-season' populations of *M. privata* at *Surrey Hills* and Tarraleah were estimated at 10.6°C and 11.9°C from mean daily temperatures during December at Waratah and Tarraleah respectively (Australian Bureau of Meteorology, 1998). Furthermore, Gilbert and Raworth (1996) noted that insects which remain active during the winter months generally have developmental thresholds roughly equal to the lowest daily average temperature in winter. Using this method, the lower developmental threshold of 'cold-season' populations of *M. privata* in southern Australia is estimated at between 7.7 and 10.3°C from daily average temperatures during July in some areas where *M. privata* is found (Table 3.1). Since the objective of this thesis was to investigate the phenology of *M. privata* in some detail, traditional temperature-rate studies were chosen over this alternative method.

Phenology models relating insect development times to temperature are subject to many sources of error, such as intraspecific variation in the temperature response within and between populations, host plant quality for phytophagous insects, thermoregulatory behavior of individuals and the presence or absence of a diapause during insect development. Intra-specific variability in the temperature response was found in the pea aphid, *Acyrthosiphon pisum* (Harris), and the cabbage

Table 3.1 Developmental thresholds of *M. privata* estimated from mean daily temperatures during mid-winter (July) for selected localities in Tasmania and mainland Australia (Source: Australian Bureau of Meteorology, 1998).

Locality [a]		Elevation (m ASL)	Mean daily minimum °C	Mean daily maximum °C	Predicted Developmental Threshold [b]
Hobart	Tas.	50	4.5	11.6	8.1
Altona	Vic.	18	4.9	13.5	9.2
Tatura	Vic.	114	2.9	12.8	7.9
Cobram	Vic.	110	2.5	12.9	7.7
Mt. Gambier	S.A.	63	5.1	13.1	9.1
Mt. Barker	W.A.	280	5.9	14.3	10.1
Manjimup	W.A.	280	6.4	14.2	10.3

[a] The list only contains localities where *M. privata* has been reported, or was found during this study.
[b] Mean daily temperature is the average of the minimum and maximum temperatures.

aphid, *Brevicoryne brassicae* (L.), which both have higher developmental thresholds in warmer climates than in cooler climates (Campbell *et al.*, 1974). Mean larval and pupal duration (at 20°C) of the fall webworm, *H. cunea,* generally increased with the latitude at which insects were collected (Gomi & Takeda, 1996).

Within a population each individual's temperature response often changes as it develops (Gordon, 1984), with different developmental stages having different temperature requirements. For example, the developmental threshold of the dragonfly *Lestes eurinus* (Say) is higher in late-instar larvae than in early instars (Lutz, 1968; cited in Danks, 1994a). Young larvae catch up to older larvae because they (young larvae) cease development later during autumn and resume development earlier in spring. Hence, adult emergence is relatively synchronous the following summer. In contrast, threshold temperatures of the cabbage white butterfly *Pieris rapae* L. (Lepidoptera: Pieridae) fall from 10.9°C for the first two instars to 7.1°C and 5.6°C in the third and fourth instars respectively (Campbell *et al.*, 1974). Campbell *et al.* (1974) suggested that the lower thresholds of later instars of *P. rapae* ensure that declining temperatures do not prevent completion of the generation before winter. Other species in which the developmental threshold changes with life-history stage include the gypsy moth (Logan *et al.*, 1991), nine species of staphylinid

beetles (Coleoptera: Staphylinidae) and three species of cholevid beetles (Coleoptera: Cholevidae) (Topp, 1994) and the chestnut weevil *Curculio elephas* Gyllenhal (Coleoptera: Curculionidae) (Manel & Debouzie, 1997).

The predictive power of a developmental model may be affected by insect thermoregulatory behaviour such as sheltering or basking, which alter an insect's microhabitat (Allsopp *et al.*, 1990; Danks, 1994a). For example, Knapp and Casey (1986) classified gypsy moth larvae as 'thermal conformers' because they seek shelter during the day and maintain a body temperature very similar to the ambient air temperature. Despite this, gypsy moth larvae developed up to three weeks faster during outbreaks because of altered larval behaviour (Lance *et al.*, 1987). Outbreak larvae remained in the canopy, whereas non-outbreak larvae sheltered below the canopy in the shade. As a result of the different behaviour, outbreak larvae were more exposed to sunlight and could be 2-6°C warmer than shaded larvae. Greater exposure to sunlight, influenced by larval density, promoted faster larval development in outbreak areas (Lance *et al.*, 1987). As *M. privata* larvae usually avoid direct sunlight during the day by remaining inside leaf shelters (Elliott & Bashford, 1978), perhaps behavioural changes during outbreaks can lead to faster than expected development of *M. privata* larvae as well. Alternatively, during outbreaks larvae may simply run out of leaves with which to make shelters or they may be forced onto adult foliage which is too resilient to fold into shelters. In this situation there would probably not be a behavioural change involved, simply a shortage of resources.

In contrast to the previous example, development time may be much slower than predicted by degree-day models if a period of diapause (discussed in Section 1.3.2) interrupts normal development. Finally, differences in foliage quality can affect the development time of feeding stages of phytophagous insects. For instance,

shorter development times and lower larval weights of winter moth larvae reared at 15°C were associated with older foliage (Topp & Kirsten, 1991), although the lower larval weight could have been due to reduced feeding time as well as foliage quality.

In conclusion, the possibility that factors other than temperature may influence development times must be considered in degree-day models otherwise development times may be shorter or longer than predicted by temperature alone. These potential sources of variation highlight that any model should be field tested and used conservatively.

3.2 AIMS AND OBJECTIVES

During pilot studies of adult emergence patterns among pupae reared from field-collected larvae (Chapter 2), pupal duration of *M. privata* was found to be highly variable: some adults emerged 40-60 days after pupation while others emerged more than 200 days after pupation. Because of such variability in the duration of the pupal stage, it was hypothesised that *M. privata* pupae had the capacity to enter a diapause, probably in response to short daylengths experienced during larval development. To test this hypothesis, an objective was set to rear *M. privata* from eggs to the pupal stage under different temperature and lighting conditions in the laboratory, and then to adult emergence at a standard temperature of 15°C (which Chapter 2 results indicate was favourable for pupal development). Short pupal periods following long daylengths and extended pupal periods following short daylengths would provide support for the diapause hypothesis. A second objective while rearing *M. privata* in the laboratory was to determine the lower temperature threshold and number of degree-days required for each immature stage in the life-cycle so that it would be possible to predict the development time for an entire generation, or any part of it, from local average temperatures. Hence, the laboratory rearing of *M. privata*

reported in this chapter had two specific objectives: (i) to relate development times of immature stages of *M. privata* to temperature, and (ii) to produce laboratory cohorts of pupae at different temperatures and daylengths for the purpose of testing the diapause hypothesis. This chapter deals exclusively with the temperature-related development of the immature stages of *M. privata*, thereby leaving the following chapter (Chapter 4) to verify or disprove the diapause hypothesis.

3.3 METHODS

A series of laboratory experiments investigated the temperature requirements for all immature stages in the life cycle of *M. privata*. Each experiment in the series addressed specific questions and built on the results of earlier experiments in the series. A pilot study on egg development (Experiment 1) was followed by further studies which investigated the temperature requirements for egg and larval stages (Experiments 2-3). As the pupae produced from these egg and larval rearing studies were subsequently reared to adult eclosion at a standard temperature of 15°C, the temperature requirements for the pupal stage (Experiment 4) were investigated using pupae reared from larvae collected in the field.

3.3.1 General rearing methods

Ovipositing females were held in plastic cages at 15°C (16L:8D) and given a juvenile shoot of *E. nitens* on which to oviposit (Fig. 3.1). A sugar solution was made by dissolving a quarter teaspoon of white sugar in 2.5 ml of water and supplied to moths in a shallow container of vermiculite. Despite previous reports that adults do not feed (Forestry Commission Tasmania, 1977), they readily extended their haustellum and fed on the sugar solution when they came into contact with it. This method of feeding enabled some moths to live for 2-4 weeks instead of the usual seven days at 15°C if given water but no carbohydrate (pers. obs.).

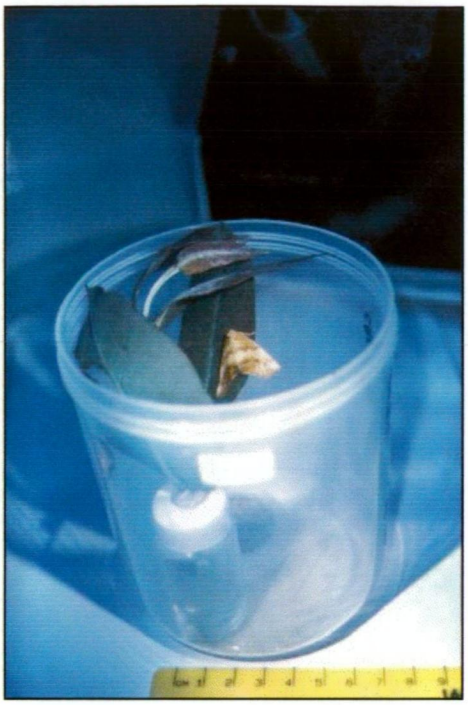
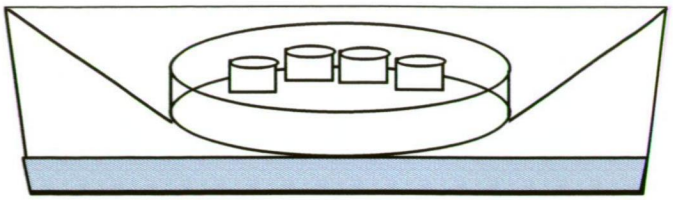


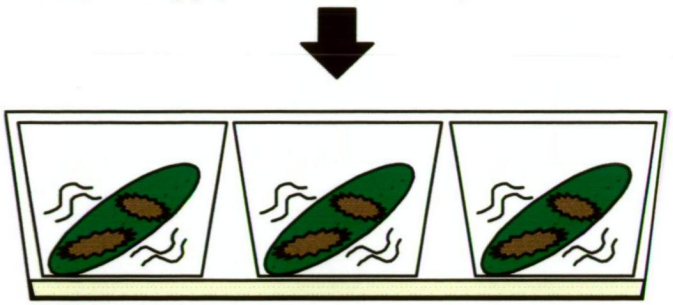
Figure 3.1 *Mnesampela privata* female resting on a *Eucalyptus nitens* shoot inside a cylindrical plastic cage. Scale divisions indicate 1 cm.

Eggs were collected each morning after oviposition and allocated to different rearing temperatures. Eggs were reared in small, inverted vial-lids (10 mm diameter, 9 mm deep) in plastic Petrie dishes (90 mm diameter, 12 mm deep) inside closed, 500 ml transparent plastic trays (Fig. 3.2a). The trays either had water in the bottom, or were lined with damp paper towel to minimize desiccation stress. When pharate neonates were visible through the transparent chorion (Fig. 3.3) the vial lids were sealed with a piece of Parafilm® (American National Can, Greenwich CT) to prevent impending neonates from escaping. Pharate neonates were checked for eclosion between one and four times daily depending on the experiment and the temperature.

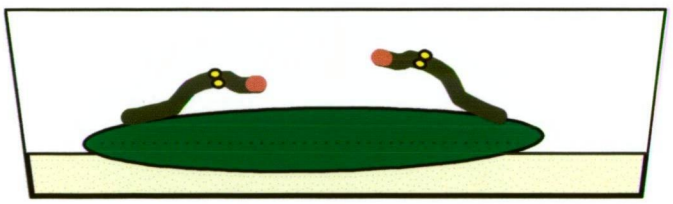
After eclosion, young larvae were reared in the vial lids until they reached the middle of the second instar. Thereafter, larvae were moved to larger rearing containers as they grew (Fig. 3.2). Eggs and larvae were reared in sibling-groups, usually ten individuals from the same egg batch, rather than individually to maintain



a) Eggs - mid L2 in vial lids in Petrie dish over water or damp paper towel in plastic trays.



b) Mid L2 - end L4 in plastic rearing cups over damp paper towel in plastic trays.



c) L5 reared in plastic rearing trays over damp paper towel.

Figure 3.2 Rearing methods used for a) eggs and early larval instars; b) middle instars; c) final instar larvae of *M. privata*.

Figure 3.3 Pharate neonates of *M. privata* clearly visible through transparent egg shells. Empty eggs have hatched.

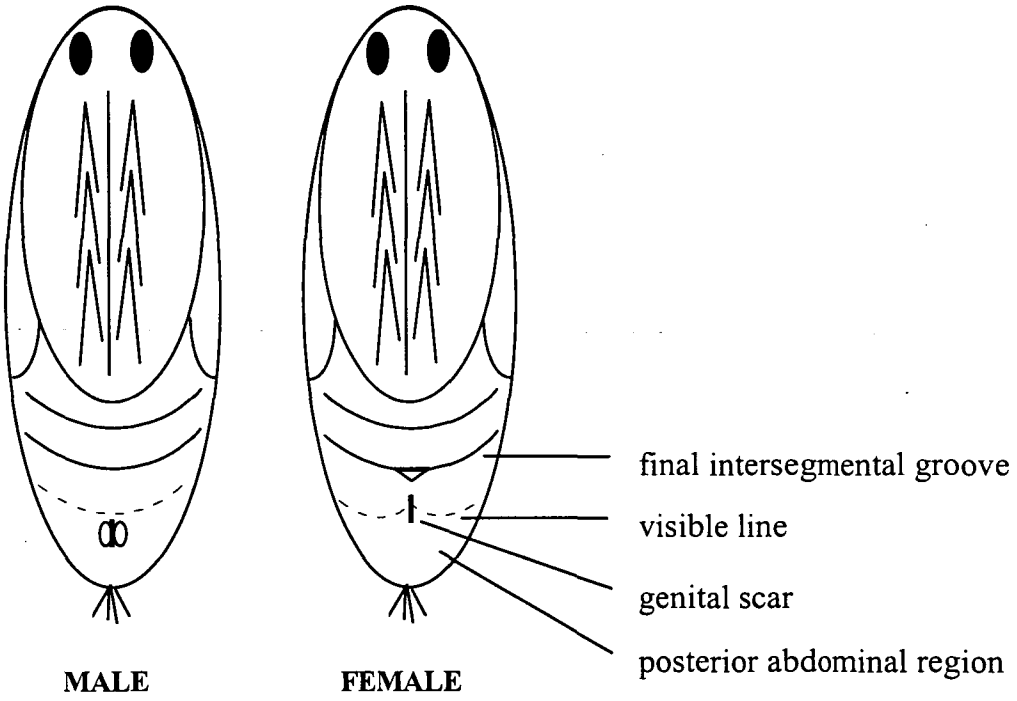


Figure 3.4 The sex of *M. privata* pupae was determined by observing the genital region under a stereo-microscope. Both sexes have a genital scar on the ventral abdominal surface posterior to the final intersegmental groove. In males the scar is situated between two small, oval shaped, slightly raised areas on the cuticle and posterior to a distinct and relatively straight visible line across the cuticle. In females the genital scar bisects and appears to draw up the visible line. Females also have a small, triangular-shaped feature beneath the final intersegmental groove which points to the genital scar. (Sexing method kindly provided by R. Schumacher, pers. comm.).

the gregarious habit of *M. privata*. Larvae were fed only on juvenile *E. nitens* foliage. During larval development, pre-moult larvae were recognized by head capsule characteristics. Several days before moulting the old head capsule resembles a small helmet worn over the larger, developing head capsule of the next instar. Pre-moult larvae were checked between one and three times daily depending on temperature, as described in the methods sections of the larval rearing experiments. The duration of each instar was the interval between two successive moults. Prepupal larvae pupated on the bottom of the rearing trays. Following pupation, pupal sex was determined by observing the genital region under a stereo-microscope (see Fig. 3.4). Pupae were then weighed, placed in inverted vial lids (25 mm

diameter; 10 mm deep) and partially covered with vermiculite. Pupae in vial lids were held in 500 ml transparent plastic trays and occasionally mist-sprayed with water to reduce pupal desiccation.

3.3.2 Experiment 1: Degree-days required for egg development

A pilot study was designed to investigate the effect of temperature, laying order and geographic origin on the rate of egg development. Key questions were:

1. What is the lower developmental threshold for egg development and how many degree-days are required for eggs to hatch?
2. Do eggs laid on different days by the same female have uniform temperature requirements?
3. Do eggs laid by females from different localities have uniform temperature requirements?

Method: Six-hundred and forty-eight eggs laid by two females from different populations in Tasmania were reared at six constant temperatures: 10, 15, 20 or 22, 25 and 30°C (Table 3.2), all under a 16hr photoperiod. One female was from *Surrey Hills* in NW Tasmania while the other was from Hobart in SE Tasmania (see Fig. 2.2). Hence, individuals from two populations over 300 km apart and from opposite ends of the state of Tasmania were represented in the pilot study on egg development.

Eggs were laid on six days during the first week of each female's oviposition period, although 18 days elapsed between first oviposition by the different females (Table 3.2). Eggs were collected each morning after oviposition and allocated evenly between five constant rearing temperatures (Table 3.2). The progeny of the two females were reared separately. Eggs were reared as previously described (Fig. 3.2a), although Petrie dishes were suspended over a saturated NaCl solution to

Table 3.2 The number of eggs allocated to each rearing temperature in the pilot study. Eggs were laid on six nights by each female and transferred to rearing temperatures the following morning.

Female	Oviposition Date (1995)	Daily Cohort	No. Eggs	Constant Temperature (°C) 10	15	20	22	25	30
Surrey Hills	19 Oct	1	74	15	15	-	14	15	15
	21 Oct	2	26	6	6	-	7	7	-
	22 Oct	3	25	5	5	-	5	5	5
	23 Oct	4	61	12	12	-	12	12	13
	24 Oct	5	58	11	12	-	12	12	11
	25 Oct	6	63	13	13	-	13	12	12
Total			307	63	64	-	62	62	56
Hobart	6 Nov	1	103	18	20	18	-	27[a]	20
	7 Nov	2	93	18	21	18	-	18	18
	8 Nov	3	34	7	7	7	-	7	6
	9 Nov	4	49	10	10	10	-	10	9
	10 Nov	5	43	8	9	9	-	9	8
	11 Nov	6	19	4	4	4	-	4	3
Total			341	65	71	66	-	75	64
Grand Total			648	128	135	66	62	137	120

[a] Seven extra eggs were found on the underside of the leaf piece containing 20 eggs originally transferred to 25°C.

maintain 75% relative humidity (Winston & Bates, 1960). Pharate neonates were checked daily for larval eclosion at all temperatures. Development times of eggs were recorded on emergence of neonates and egg development rates were calculated from the reciprocals of development times. A mean development rate was calculated for each female x temperature x day factor combination in Table 3.2, then multiplied by 100 to express mean development rate as a daily percentage. For instance, mean development rates for eggs laid by the *Surrey Hills* female on 19.Oct.1995 were based on 14-15 eggs at each temperature, whereas development rates for eggs laid on 22.Oct.1995 were based on five eggs at each temperature (Table 3.2), assuming no mortality. Using the mean response of related individuals in a temperature x day factor combination avoided pseudo-replication, which would occur if independent values were taken from related individuals (Potvin, 1993; Watt, 1993).

Statistical analysis: The effects of temperature, female and day of oviposition on egg development rate were quantified by simple linear regression with groups (Genstat 5 Release 3.2, 1993). Development rate was the dependent variable, temperature was the independent variable, while female and day of oviposition were the grouping factors. This method of analysis was chosen as it tests whether or not different groups, i.e. eggs laid on different days or by different females, have significantly different regression lines (as shown schematically in Fig. 3.5). If groups have significantly different regression lines, regression equations must be determined for each group, whereas groups found to have statistically similar regression lines may be pooled to determine an overall regression equation. Lower developmental thresholds and degree-day requirements can then be estimated from the coefficients (α and β) of the final regression equation(s).

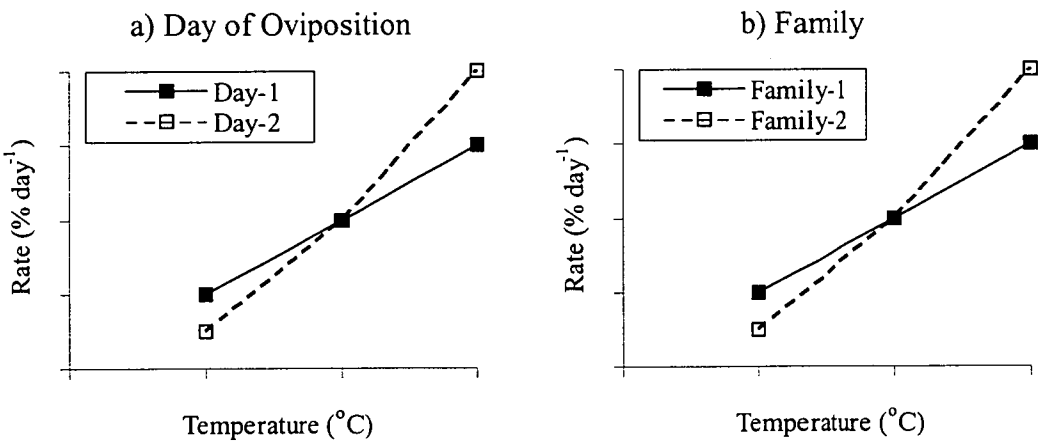


Figure 3.5 Schematic diagram representing different regression lines associated with (a) different days of oviposition, and (b) different families. The term 'family' refers to the progeny of a single female moth.

3.3.3 Experiment 2. Intra-specific variation in egg development rates

Experiment 2 was designed primarily to investigate intra-specific variation in the temperature response of *M. privata* eggs, but also to test for variability in larval development rates at two temperatures while establishing suitable larval rearing methods. Key questions were:

1. Do egg and/or larval development rates vary among different families?
2. What is the lower temperature threshold for larval development and how many degree-days are required to complete the larval stage?

Method: In Experiment 2, 1727 eggs laid by 14 females over two consecutive nights, 23rd and 24th January 1996, were reared at one fluctuating temperature of 25/15°C (8L:16D), equivalent to a mean daily temperature of 18.3°C, and five constant temperatures of 10, 15, 20, 23 and 30°C. A 16 hr daylength was used at each constant temperature except 20°C, which had an 8 hr daylength. Eggs were laid by ten females each night, with six females ovipositing on both nights, four females ovipositing on 23 January only and a further four females ovipositing on 24 January only (Fig. 3.6). The progeny of each female were reared separately from the progeny of other females. The bulk of the eggs (n=1514) were laid by 12 wild females hand collected at dawn on 19th January 1996 from the ground and trees surrounding two black-light traps placed in high-altitude *E. nitens* plantations 5 and 20 km south of Tarraleah the previous evening. Adults were held at 4°C for two days while they were sorted. Thirty-two females from Tarraleah were originally held in plastic oviposition chambers, but only 12 females laid enough eggs (≥40 eggs on at least one of the two nights) to be included in the experiment. The remaining 213 eggs were laid by two *Surrey Hills* females that emerged in the laboratory several days before the experiment began.

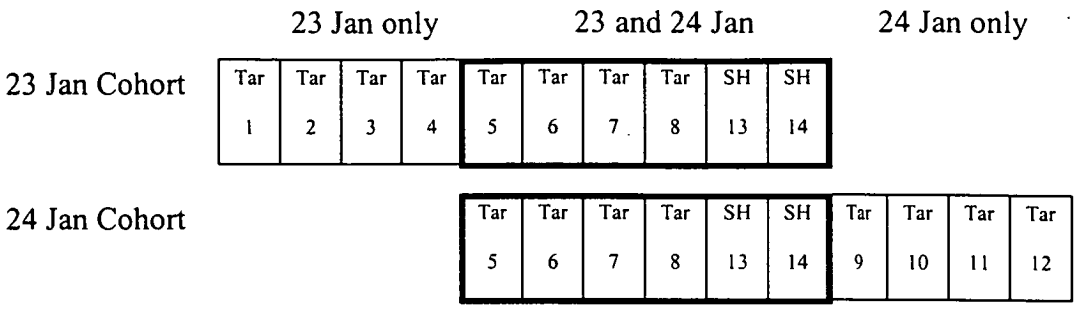


Figure 3.6 Eggs from 14 families (numbered 1-14) were laid over two evenings. Six females laid eggs on both days, while eight females laid eggs on only one of the two days. Twelve families were from Tarraleah (Tar) in central Tasmania and two were from *Surrey Hills* (SH) in NW Tasmania.

Most eggs (n=1480) were allocated to the four intermediate temperatures (15, 18.3, 20 and 23°C) as the pilot study found that eggs at 30°C died and that development at 10°C was relatively slow, taking double the time required at 15°C. Each morning after oviposition, between 10 and 30 eggs per female (except the two *Surrey Hills* females) were allocated to each of the four main temperatures. Between 6 and 20 eggs from each *Surrey Hills* female were allocated to rearing temperatures each morning as those two females laid fewer eggs. Eggs were allocated to 10 and 30°C when surplus eggs were available. The allocation of eggs took 3-4 hours each morning, but was completed by about 1pm on the day after oviposition. Assuming an oviposition time of 10 pm the previous evening (the middle of the 8 hr dark period in the incubator), eggs were assumed to have spent 15 hrs at 15°C prior to the allocation. Pharate neonates were checked for larval eclosion daily at 10°C, twice daily at 15°C and every 6-8 hrs above 15°C. Neonates that hatched at 15 and 20°C were subsequently reared as far as possible at those two temperatures to establish suitable larval rearing methods. Larval groups recognised as pre-moult by head-capsule characteristics were checked every 12 hours (9 am, 9 pm) at both rearing temperatures and the number of larvae in each instar was recorded. The larval pilot

study was terminated after larvae reached the 4th and 5th instars at 15 and 20°C respectively as larval mortality was very high (see results).

Statistical analysis: At each temperature, mean development rates of eggs were determined separately for family groups from each day of oviposition. Development rate was then regressed against temperature in order to determine T_0 and K as before. Development rate was the dependent variable, temperature was the independent variable, while day of oviposition and family were used as grouping factors. As the larval pilot study was cut short due to high larval mortality, T_0 and K required to complete the larval stage could not be estimated. However, despite not achieving the original objective, preliminary estimates of T_0 and K required by *M. privata* to develop from fresh eggs to the fourth instar, hereafter called 'time-to-L4', were obtained by plotting the overall development rate between oviposition and L4 against temperature, albeit at just two temperatures. For practical purposes, time-to-L4 is important as it represents the window of opportunity for population control before significant larval feeding damage begins. Hence, useful information was still gathered despite terminating the larval pilot study before larvae pupated.

3.3.4 Experiment 3: Degree-days required for larval development

Experiment 3 was designed to rear *M. privata* from fresh eggs to the pupal stage at more than two temperatures so that the temperature requirements of the entire larval stage could be determined. Furthermore, the opportunity arose to do a comparative study of development rates between families from Tasmania and mainland Australia. Key questions were:

1. What is the threshold temperature for larval development and how many degree-days are required to complete the larval stage?
2. Are the temperature requirements of Tasmanian and mainland families similar?

Methods: The progeny of two females were used in Experiment 3. One female originated from Tarraleah in central Tasmania, the other from Cobram in northern Victoria (Fig. 3.7), although both females emerged and were mated in the laboratory. The Cobram female was reared from larvae collected there on 7 March 1996 while the Tarraleah female was reared from the surviving larvae in Experiment 2. A total of 317 eggs laid by the Tarraleah female over four consecutive nights, 19-22 June 1996, and 150 eggs laid by the Cobram female on one night, 24th June 1996, were reared under the temperature and lighting conditions in Table 3.3. Larvae that hatched from eggs were reared to pupation at their respective hatching temperatures.

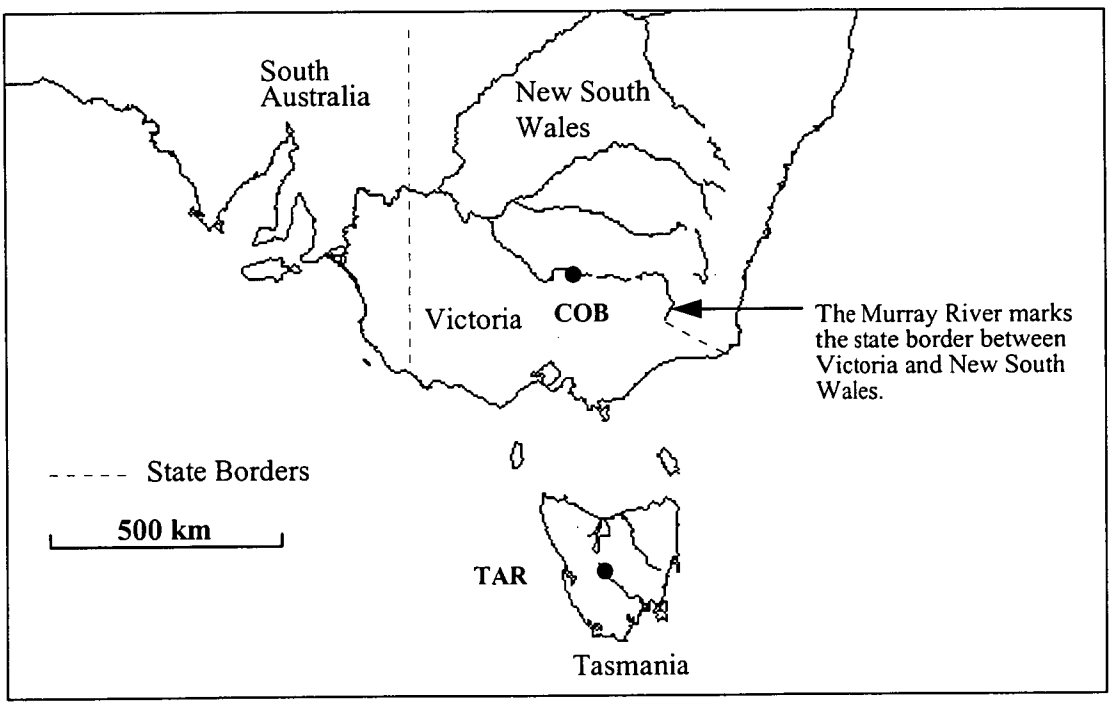


Figure 3.7 The geographic origin of the two *M. privata* families used in Experiment 3. The Tasmanian family originated from Tarraleah (TAR), while the mainland family originated from Cobram (COB) near the Victoria-New South Wales border.

Table 3.3 Temperatures and lighting conditions under which *M. privata* eggs and larvae were reared in Experiment 3.

Temperature (°C)	11.5	15.0	16.8	24/18 [a]	24.0
Lighting (L:D)	16:8	16:8	8:16	12:12	0:24

[a] The mean daily temperature of the fluctuating regime was 21°C.

Eggs and larvae were reared as described previously in Section 3.3.1. One problem encountered in the larval pilot study was that foliage pieces desiccated after 1-2 days at 20°C. A contributing factor may have been that excised leaf pieces lost water more rapidly when illuminated because of transpiration. To overcome the problem of foliage desiccation at higher temperatures, fresh foliage was added daily at both 24/18°C and 24°C and larvae were reared in darkness at 24°C to reduce transpirational water loss by excised leaves. Pharate neonates were checked twice daily (9 am, 6 pm) for hatching at 11.5, 15 and 16.8°C, and every eight hours (4 am, 12 noon, and 8 pm) at 24/18°C and 24°C. At 11.5°C all larval stages were checked daily for moulting to the next instar. At 15 and 16.8°C instars I-III were checked twice daily (9 am, 6 pm), remaining instars daily. At 24/18°C all instars were checked twice daily until pupation, while at 24°C instars I-II were checked every eight hours, remaining instars twice daily.

Statistical analysis: At each temperature, mean development rates of eggs and each larval instar were determined separately for each day of oviposition. A general linear regression model containing terms for development rate (the dependent variable), temperature (the independent variable), developmental stage and day of oviposition (the grouping factors) was used to examine whether regression lines varied from stage to stage and for different days of oviposition. As the Tarraleah female oviposited on 19-22 June and the Cobram female oviposited on 24th June 1996, the term for day of oviposition was deemed to test for day effects and family effects

simultaneously. For instance, if day effects were not significant, family effects were also deemed to be not significant, whereas significant day effects might indicate developmental differences between the two families. Overall development rates between oviposition and pupation (time-to-pupa) were also determined and regressed against temperature in order to estimate T_0 and K required for the entire egg-larval period.

3.3.5 Experiment 4: Degree-days required for pupal development

Experiment 4 was designed to quantify the effect of temperature on the period of adult differentiation, i.e. the period between the onset of pupal development and adult eclosion. It must be remembered that, as found in Chapter 2, pupae may remain dormant, probably in diapause, for many months at temperatures favourable for development before adult differentiation begins. Thus, these experiments were not designed to measure the whole pupal period (resting and/or quiescent period + pupal development), but only the pupal development period. Pupal diapause is dealt with in Chapter 4. Key questions were:

1. What is the lower temperature threshold for adult differentiation?
2. How many degree-days are required for adult differentiation to be completed?

Answering these questions for the pupal stage would be the last step in determining the physiological generation time of *M. privata* in the absence of any developmental interruptions brought about by either quiescence or pupal diapause.

Methods:

Pupal development at 11.5 to 24 °C

Ninety-two newly-developing pupae were placed at the constant and fluctuating temperatures given in Table 3.4. The onset of pupal development was recognised by a dorsal shift in the position of eye-spots in 44 pupae and by the presence of developing ommatidia in the eye region of the pupal head in a further 48 pupae.

Table 3.4 Temperatures at which *M. privata* pupae were placed after the start of adult differentiation.

Mean Daily Temperature (°C)	11.5	15.0	17.5	20.0	21.0	23.8	24.0
Range if fluctuating temperature (°C)			18 / 16		24 / 18	25 / 20	
Thermoperiod (hrs)	24	24	18 : 6	24	12 : 12	18 : 6	24
No. of pupae at each temperature	21	18	10	5	15	8	15

Despite the fact that pupae with developing ommatidia were slightly more developed than pupae with shifted eye-spots, in order to simplify the analysis all pupae were deemed to be at the same state of development when placed at the various rearing temperatures. The difference in starting times is estimated to be less than 7 days, or 13% of total pupal development time of about 54 days at 15°C (found in Chapter 2). As the timing of onset of pupal development was highly variable, at no time were large numbers of newly-developing pupae available simultaneously for use in developmental studies. Consequently, it was necessary to place small groups of newly-developing pupae at different rearing temperatures as pupae became available over a two year period, then compile the results to determine the overall relationship between pupal development rate and temperature.

At each temperature, the state of pupal development was determined periodically under a stereo-microscope, but otherwise, pupae remained in darkness. Each checking period lasted only a few minutes. On each examination, the developmental state of a pupa was classified as either: (1) eye-spots shifted but still visible; (2) ommatidia visible and body clouded; (3) black eye phase; (4) unpigmented pharate adult; (5) wing pigmentation visible; or (6) leg-pigmentation visible, which was deemed to be pre-eclosion. These six developmental states are represented schematically in Figure 3.8. Pupae at 11.5 and 15°C were usually checked twice weekly until they reached the pre-eclosion state, after which they were

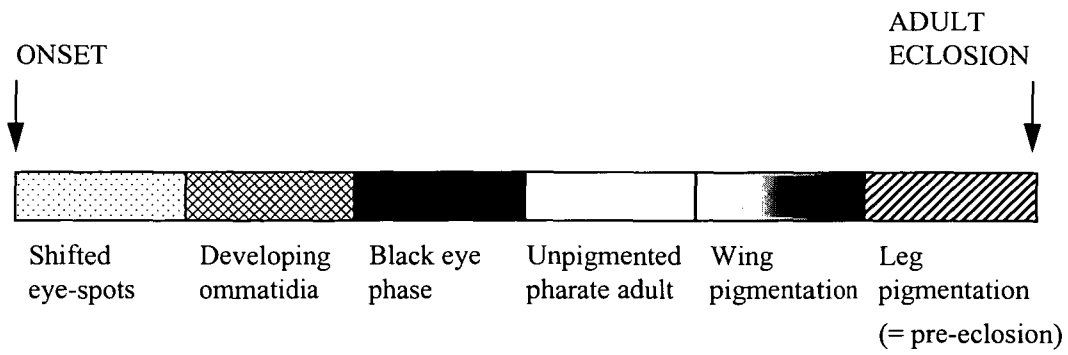


Figure 3.8 Schematic diagram of six states of development recognised in *M. privata* pupae during adult differentiation.

checked daily for adult eclosion, while pupae reared at temperatures above 15°C were usually checked 3-4 times weekly until they reached the pre-eclosion state, then daily until adult eclosion. Overall development rates were calculated at each temperature from the reciprocals of development times (in days) then transformed to daily percentages. The relationship between pupal development rate and temperature was then investigated using regression analysis, from which T_0 and K required for adult differentiation were estimated. Also, after finding that adult differentiation could be divided into two distinct stages on the basis of different temperature responses (see results), the temperature requirements for each stage were investigated separately using regression analysis.

Effect of high temperatures on pupal development

Pupae were originally placed at temperatures ranging from 11.5 to 24°C (Table 3.4) because those temperatures were found to be favourable for egg and larval development. However, as *M. privata* routinely oversummers in the pupal stage in most areas of its distribution, an additional 41 newly-developing pupae were subsequently placed at 26, 30 or 35°C to extend the author's understanding of high temperature effects on pupal development. Earlier results from pupae placed at

20-24°C suggested that high temperatures inhibited development of the pharate adult during the second half of adult differentiation (see results). Consequently, half the pupae placed at each high temperature (26-35°C) were transferred to 15°C when they became unpigmented pharate adults while the other half remained at high temperatures (Table 3.5). The mean number of days required to develop: (a) from onset of adult differentiation to an unpigmented pharate adult; (b) from an unpigmented pharate adult to adult eclosion; and (c) from onset to eclosion were determined for each of the six treatments in Table 3.5. T-tests were then used to determine whether pupae transferred to 15°C half-way through adult differentiation completed development significantly faster than control pupae remaining at high temperatures.

Table 3.5 A summary of the six treatments used to investigate the effect of high temperature on the development of *M. privata* pupae.

<table>
<tr><th>Hypotheses</th><th colspan="2">Treatments</th></tr>
<tr><td rowspan="7">1. High temperatures inhibit development of the pharate adult during the second half of adult differentiation.

2. Unpigmented pharate adults transferred to 15°C will complete development more rapidly than controls remaining at high temperatures.</td><td colspan="2">Pupae were reared to an unpigmented pharate adult at 26, 30 and 35°C. Then, half the pupae at each temperature were transferred to 15°C, while the other half remained as high-temperature controls, as indicated by the six treatments below:</td></tr>
<tr><td>Treatment No.
(No. of pupae)</td><td>Temperatures during adult differentiation (°C)
First half : Second half</td></tr>
<tr><td>1 (7)
2 (7)</td><td>26 : 26
26 : 15</td></tr>
<tr><td>3 (7)
4 (6)</td><td>30 : 30
30 : 15</td></tr>
<tr><td>5 (7)
6 (7)</td><td>35 : 35
35 : 15</td></tr>
</table>

3.4 RESULTS

3.4.1 Experiment 1: Degree-days required for egg development

The relationship between *M. privata* egg development rate and temperature is presented in Figure 3.9. There was a strong linear relationship between egg development rate and temperature between 10 and 25°C, but at 30°C all eggs died. Some embryonic development was observed at 30°C, but developing embryos died and desiccated inside the chorion well before the pharate neonate stage. Since all eggs died at 30°C, that temperature treatment was excluded from statistical analyses. Also, as the oviposition periods of the *Surrey Hills* and Hobart females did not overlap, the effect of laying order (i.e. day of oviposition) on egg development rate was examined first in the *Surrey Hills* family and then in the Hobart family.

Temperature was the overwhelming factor to affect egg development rate in the *Surrey Hills* family, explaining 99.6% of total variation. Eggs laid on six days had a uniform temperature response, as the regression slopes ($F_{5,12} = 0.53$, $p = 0.75$) and intercepts ($F_{5,17} = 1.55$, $p = 0.23$) corresponding to the six days of oviposition were not significantly different. Similarly, temperature explained 99.4% of total variation in egg development rates in the Hobart family, with eggs laid over six days by the Hobart female also having a uniform temperature response: slope test ($F_{5,9} = 0.18$, $p = 0.96$); intercept test ($F_{5,14} = 0.52$, $p = 0.76$). Thus, laying order had no effect on egg development rate in either family, meaning that daily sibling-groups at each temperature could be considered replicates and pooled to compare the temperature responses of the two families. Regression slopes ($F_{1,41} = 2.95$, $p = 0.09$) and intercepts ($F_{1,42} = 0.14$, $p = 0.71$) of the two families were also not significantly different, indicating that both families had very similar temperature requirements for egg development. Finally, as there was no evidence to suggest that eggs laid on different days by different females had different temperature requirements, data from

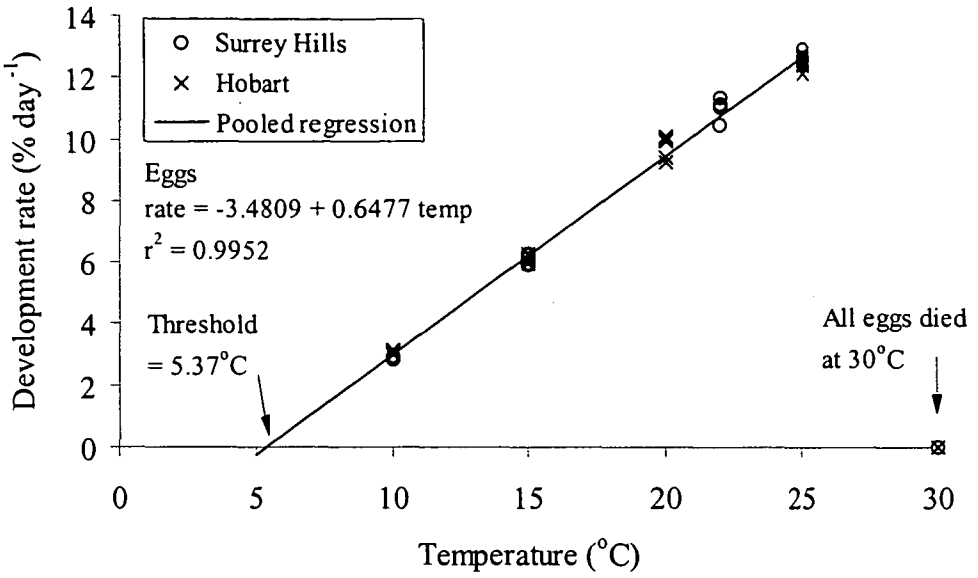


Figure 3.9 The relationship between *M. privata* egg development rate and temperature. The result is based on the progeny of two females from opposite ends of the state of Tasmania.

the two families were pooled to determine an overall regression equation for egg development rate against temperature. The final regression equation is also presented in Figure 3.9. From that regression equation, egg development rate was summarised as R = 0.6477 (T - 5.37), where R= egg development rate in % per day and T = constant temperature (Regression: F1,43 = 8901.87, $p < 0.001$; r2 = 0.995). The developmental threshold (To) was estimated at 5.37 ± 0.15 (SE) °C and 154.38 ± 1.64 DD were required for eggs to hatch. Since eggs were assumed to have spent an average of at least 12 hours at 15°C between oviposition and transfer to the rearing temperatures, K may be increased by 4.8 DD. Thus, fresh eggs of M. privata hatched when approximately 159 ± 2 DD > 5.4 ± 0.2°C were accumulated. However, as this result was based on the progeny of just two females, it was viewed as a preliminary finding to be validated by further experimentation. The next experiment in the series (Experiment 2) repeated the experiment with eggs from a larger number of females in order to examine the level of intraspecific variation in egg development rates.

Egg mortality at 10-25°C was very much lower than the 100% found at 30°C (Fig. 3.10). In the *Surrey Hills* family, egg mortality was 3.2% at 22°C and 0% at 10, 15 and 25°C, while in the Hobart family, egg mortality rate was 0% at 20°C and 1.5, 38.0 and 6.7% at 10, 15 and 25°C respectively. The 38% egg mortality at 15°C in the Hobart family was due mainly to the death of 25 out of 26 eggs from the 8.Nov, 9.Nov, and 10.Nov groups. Those eggs died after their developmental stage was photographed under a stereo-microscope. It is assumed that eggs were overheated by the light source used. It is not thought that the mortality was due to

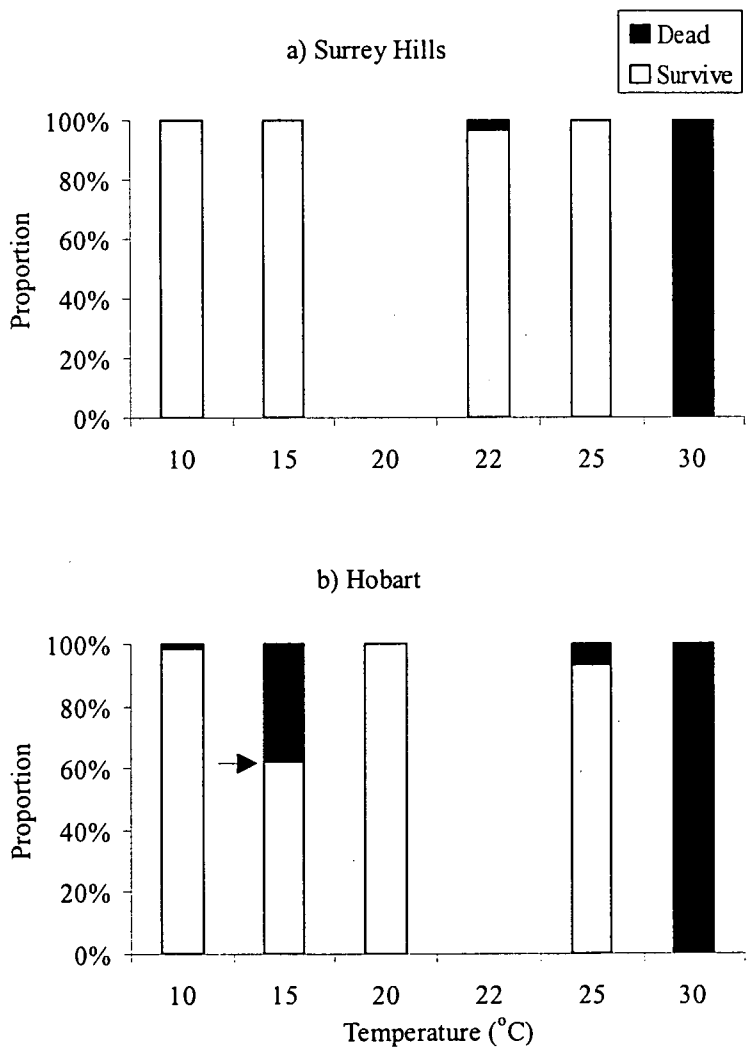


Figure 3.10 Proportion of dead and surviving eggs of *M. privata* at each constant rearing temperature between 10 and 30°C. The 38% mortality at 15°C in the Hobart family (see arrow) was an aberration caused by the overheating and subsequent death of three groups of eggs that were photographed under a microscope.

the 15°C incubation temperature. Thus, at a relative humidity of 75%, temperatures of 10-25°C were favourable for the development of *M. privata* eggs and within the linear range of the temperature-response curve for the species.

3.4.2 Experiment 2. Intra-specific variation in egg development rates

As in the pilot study on egg development, all eggs died at 30°C so that temperature was excluded from analysis. Aside from the 30°C treatment, overall egg mortality in Experiment 2 was just 1.62%, representing 26 eggs that died among the 1606 eggs transferred to temperatures between 10 and 23°C over two days. Since egg mortality was so low, the data presented on egg development rates are not biased by mortality.

The relationship between *M. privata* egg development rate and temperature in Experiment 2 is presented in Figure 3.11. Temperature was the overwhelming factor to affect egg development rates ($F_{1,49} = 2528.85$, $p < 0.001$), and explained 97.4% of all variation. Family effects were negligible ($F_{13,49} = 0.39$, $p = 0.967$) indicating that the temperature requirements of all 14 families were uniform. Hence, for practical purposes, intraspecific variation in egg development rates need not be considered. The similarity in mean egg development rates within a temperature is illustrated in Table 3.6, which presents the mean development rates for ten families at four incubation temperatures. Day of oviposition ($F_{1,49} = 1.47$, $p = 0.231$) and all interaction terms were also not significant. Therefore, as the grouping factors had no significant effects on development rates, the data were re-analysed after dropping family and day of oviposition from the model. The final regression equation, based on the pooled results from 14 families and two days of oviposition, is also presented in Figure 3.11. From that regression equation, the developmental threshold was estimated at 6.23 ± 0.22°C, above which 146.48 ± 2.57 DD were required for eggs to hatch. The degree-day requirement may be increased by 5.48 DD to allow for 15 hrs

of development at 15°C before eggs were transferred to rearing temperatures. Thus, approximately 152 DD > 6.2°C were required for fresh eggs to hatch in the second experiment. This compared with estimates of 159 DD > 5.4°C in the pilot study on egg development.

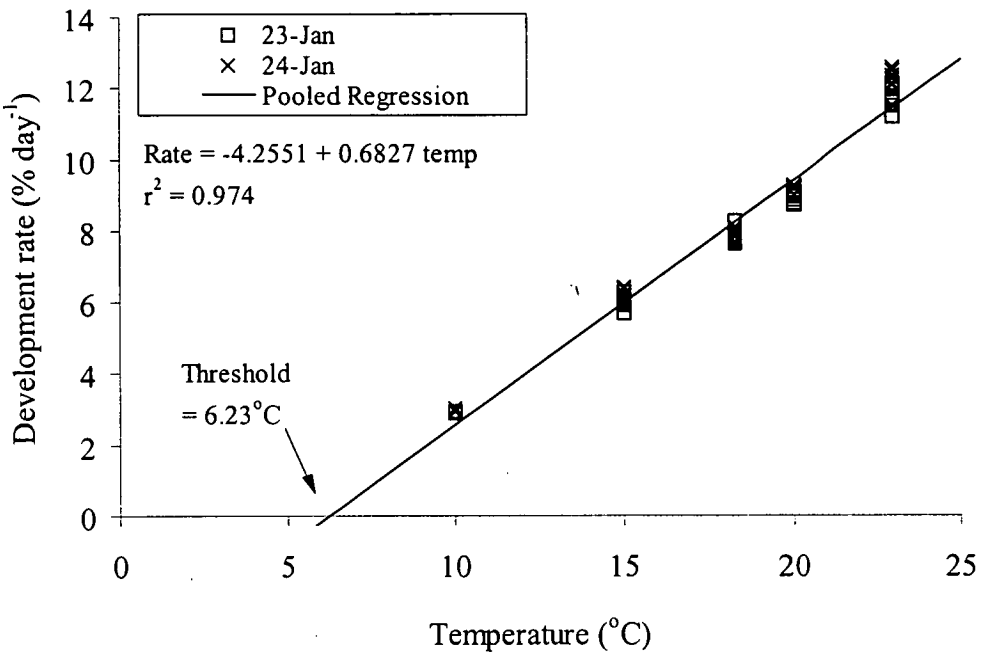


Figure 3.11 The relationship between *M. privata* egg development rate and temperature, based on the pooled results of 14 families and two nights of oviposition.

Table 3.6 Mean ± SD *M. privata* egg development rates (% day^{-1}) at four temperatures for 10 families in the first daily cohort in Experiment 2 (i.e. for eggs laid on 23 Jan 1996).

	Temperature (°C)												
	15			18.3			20			23			
Family	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Total n
Tar 1	5.97	0.05	19	8.25	0.27	20	9.04	0.08	20	11.52	0.24	20	79
Tar 2	5.99	0.00	10	7.72	0.24	10	8.94	0.18	10	11.75	0.23	10	40
Tar 3	6.08	0.15	19	7.87	0.27	20	9.01	0.00	20	12.13	0.32	20	79
Tar 4	5.70	0.10	20	7.63	0.07	20	8.74	0.25	20	11.22	0.10	20	80
Tar 5	5.93	0.10	18	7.77	0.15	20	9.02	0.05	20	11.64	0.37	20	78
Tar 6	5.96	0.12	20	7.79	0.08	20	9.09	0.14	20	11.96	0.17	20	80
Tar 7	5.98	0.06	10	7.73	0.07	10	9.07	0.10	10	11.73	0.43	10	40
Tar 8	5.99	0.00	12	7.66	0.08	10	8.84	0.25	8	11.19	0.00	10	40
SH 13	6.28	0.00	7	7.62	0.06	7	8.75	0.11	6	11.72	0.36	6	26
SH 14	6.27	0.00	8	7.62	0.07	8	8.71	0.12	9	11.76	0.55	6	31
All Families	5.98	0.17	143	7.80	0.26	145	8.95	0.19	143	11.67	0.41	142	573

Larval pilot study

The mean development times required by *M. privata* to develop from oviposition to the start of instars I-IV at 15°C and instars I-V at 20°C are presented in Table 3.7. Development times were consistently about 50% longer at 15°C than at 20°C, as indicated in the bottom row of Table 3.7. For example, development times at 15°C and 20°C respectively were around 24 and 16 days to reach L2, 30 and 20 days to reach L3 and 36 and 24 days to reach L4. As a consequence, the mean development rate between oviposition and the start of the fourth instar (i.e. time-to-L4) was around 50 percent faster at 20°C (4.11 ± 0.01 % per day) than at 15°C (2.71 ± 0.01 % per day) (Fig. 3.12).

Cumulative percentage survival during the larval pilot study is also presented in Table 3.7. Survival rates from oviposition to the start of the third instar were similar at both temperatures, with more than 97% of eggs hatching, around 80% survival to L2 and over 70% survival to L3 (Table 3.7). Thereafter, survival to the fourth instar fell to 29.5% at 15°C and 41.9% at 20°C, while survival to the fifth instar at 20°C was just 8.5%. The larval pilot study was terminated after five weeks due to this high rate of larval mortality. The rearing trial showed that larval mortality, particularly during the third and fourth instars, would need to be reduced before sufficient numbers of pupae could be produced under controlled temperature and lighting conditions for further experiments on adult phenology. Due to this high rate of larval mortality and the fact that larvae were reared at only two temperatures, no statistical comparisons were made between the development rates of the different families. However, mean development rates of daily family groups were determined at each temperature, then regressed against temperature to obtain preliminary estimates of T_0 and K required by *M. privata* to develop from oviposition

Table 3.7 Development time (days) and cumulative percentage survival (% S) of *M. privata* from oviposition to the start of L1-L4 at 15°C and L1-L5 at 20°C. The results are based on pooled data from 14 families and two days of oviposition.

	Instar																			
Temperature	L1				L2				L3				L4				L5			
°C	Mean	SD	n	% S	Mean	SD	n	% S	Mean	SD	n	% S	Mean	SD	n	% S	Mean	SD	n	% S
15	16.5	0.5	367	98.4	24.0	0.7	293	78.6	30.1	0.9	270	72.4	36.9	0.9	110	29.5	-	-	-	-
20	11.0	0.2	367	97.3	16.5	0.8	309	82.0	20.1	1.0	277	73.5	24.4	1.1	158	41.9	30.0	1.0	32	8.5
Ratio 15:20	1.50				1.46				1.50				1.52				-			

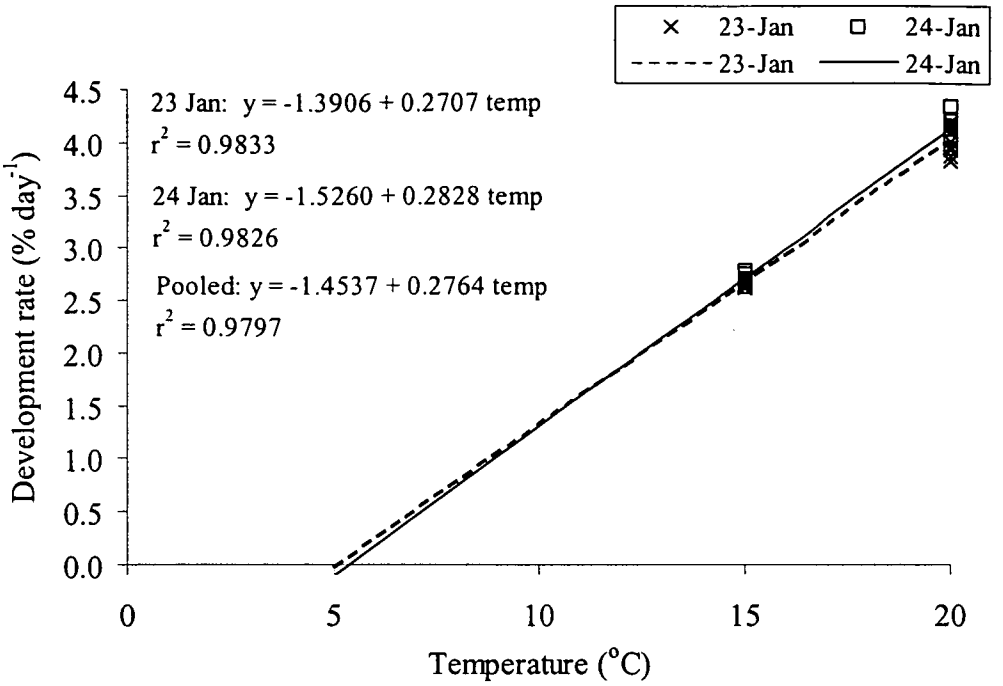


Figure 3.12 The relationship between temperature and the overall development rate of *M. privata* between oviposition and the start of the fourth instar. Each data point at 15 and 20°C is a mean development rate for one family group from one day of oviposition. Results for each day of oviposition are plotted separately, but generally overlap, suggesting no major differences between the two days.

Table 3.8 Estimated lower temperature threshold (T_0) and number of degree-days (K) required by *M. privata* to develop from oviposition to the fourth instar.

	T_0	K
Oviposition date	Mean ± SE	Mean ± SE
23 Jan	5.14 ± 0.39	369.39 ± 11.67
24 Jan	5.40 ± 0.42	353.60 ± 12.15
Pooled	5.26 ± 0.30	361.82 ± 8.93

to the fourth instar (Fig. 3.12). Preliminary estimates of T_0 and K, based on development rates at 15°C and 20°C, were 5.3°C and 362 DD respectively (Table 3.8). Although this result is based on the pooled results from 14 families and two days of oviposition, reference to Figure 3.12 suggests that mean development rates of daily family groups did not vary greatly within a temperature.

3.4.3 Experiment 3: Degree-days required for larval development

The preliminary regression analysis determined that the temperature, stage and temperature by stage interaction terms were highly significant ($p < 0.001$), whereas day of oviposition and all interaction terms involving day of oviposition were not significant (Table 3.9). This result meant that development rates varied significantly with temperature and developmental stage, but did not vary in relation to day of oviposition (and hence family). On the basis of this result, separate regression equations of temperature versus development rate were determined for each developmental stage after pooling the data from the five days of oviposition. Regression equations for the egg and larval stages of *M. privata* are presented in Figure 3.13, while mean development times of egg and larval stages at five temperatures are presented in Table 3.10. Development rates generally increased with increasing temperature, with r^2 values for individual stages ranging from 88.5% for prepupae to 98.6% for eggs (Fig. 3.13). As a consequence, development times decreased with increasing temperature (Table 3.10). The effect of temperature on the overall development rate between oviposition and pupation (time-to-pupa) is also given in Figure 3.13. The regression line for time-to-pupa indicates a strong linear relationship between temperature and the overall rate of development between oviposition and pupation ($r^2 = 99.0\%$).

Table 3.9 Analysis of variance results from the regression of *M. privata* development rate against temperature, developmental stage and oviposition date.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
temp	1	3482.125	3482.125	1589.06	<.001
stage	6	3579.382	596.564	272.24	<.001
date	4	8.147	2.037	0.93	0.450
temp.stage	6	488.046	81.341	37.12	<.001
temp.date	4	2.333	0.583	0.27	0.899
stage.date	24	30.704	1.279	0.58	0.934
temp.stage.date	24	35.961	1.498	0.68	0.856
Residual	96	210.366	2.191		
Total	165	7837.064	47.497		

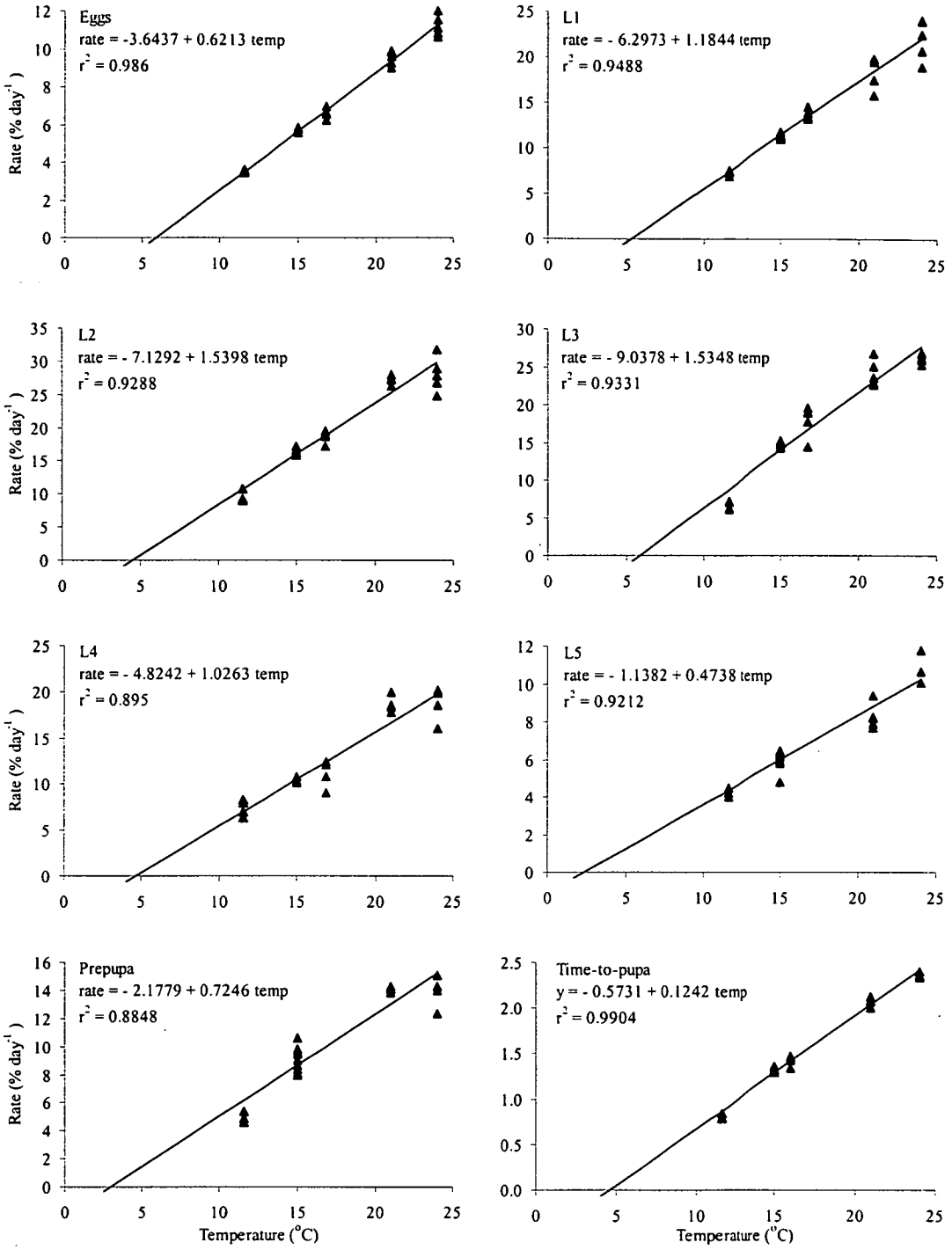


Figure 3.13 Temperature-related development rates for eggs and all larval stages of *M. privata*. Time-to-pupa represents the overall development rate between oviposition and pupation. Probability of all regressions < 0.001.

Table 3.10 Development time (days) and cumulative percentage survival (% S) of the egg and larval stages of *M. privata* at five temperatures.

	Temperature and Photophase																					
	11.5 °C (16L:8D)				15°C (16L:8D)				16.8°C (8L:16D)				24/18°C (12L:12D)				24°C (0L:24D)				Total	
Stage	Mean	SE	n	% S	Mean	SE	n	% S	Mean	SE	n	% S	Mean	SE	n	% S	Mean	SE	n	% S	n	% S
Egg	28.4	0.1	69	100.0	17.3	0.0	99	99.0	15.0	0.1	98	100.0	10.4	0.0	98	98.0	8.9	0.1	98	98.0	462	98.9
L1	13.8	0.1	54	78.3	8.8	0.1	91	91.0	7.3	0.1	88	89.8	5.4	0.1	83	83.0	4.6	0.1	77	77.0	393	84.2
L2	10.5	0.2	51	73.9	6.1	0.1	90	90.0	5.4	0.1	87	88.8	3.7	0.0	80	80.0	3.8	0.1	74	74.0	382	81.8
L3	14.7	0.2	49	71.0	6.8	0.1	88	88.0	5.8	0.1	76	77.6	4.1	0.0	79	79.0	3.9	0.1	59	59.0	351	75.2
L4	14.3	0.3	49	71.0	9.8	0.2	87	87.0	9.7	0.6	76	77.6	5.5	0.1	78	78.0	5.6	0.1	51	51.0	341	73.0
L5	23.6	0.3	48	69.9	18.1	0.6	77	77.0	16.5[a]	0.3	72	73.5	13.0	0.5	76	76.0	9.7	0.3	39	39.0	312	66.8
PP	19.6	0.3	46	66.7	10.4	0.2	69	69.0	11.9[a]	0.1	68	69.4	7.2	0.1	73	73.0	7.2	0.2	32	32.0	288	61.7
Egg + larva[b]	124.8	0.7			75.5	0.4			70.5	0.5			48.6	0.4			42.6	0.3				

[a] Larvae reared at 16.8°C were transferred to 15°C late in the fourth instar due to equipment shortages. Development times of L5 and PP at 16.8°C would therefore be slightly shorter than the periods presented in the table.

[b] The total development time for the egg-larval period (egg + larva) at each temperature is a mean based on all individuals surviving to pupation at that temperature, not the sum of values in the column above. Due to the rounding up or down of decimal places, the sum of the stages may not exactly equal the total development time presented in the table.

Estimated lower temperature thresholds and thermal constants for the egg and larval stages of *M. privata*, based on the regression equations in Figure 3.13, are presented in Table 3.11. Developmental thresholds for individual stages ranged from approximately 5.9°C for eggs and L3 down to 2.4°C for the fifth instar. Strictly speaking, the fifth instar of *M. privata* includes both an active feeding stage and an inactive, non-feeding prepupal stage. However, as the prepupal stage causes no damage, it is distinguished from the active feeding stage in both Figure 3.13 and Table 3.11. Thermal constants within an instar ranged from about 65 DD for L2 and L3 up to 211 DD for the fifth instar, although each thermal constant is based on a different base temperature (Table 3.11).

Table 3.11 Estimated lower temperature threshold ($T_0 \pm$ SE) and number of degree-days ($K \pm$ SE) required by eggs and all larval stages of *M. privata*. The last three columns refer to thermal requirements above a fixed threshold of 5°C.

Stage	T_0 (°C)	K (DD)	DD > 5°C		Proportion Spent in Each Stage (%)
			Each Stage	Cumulative	
Eggs	5.87 ± 0.33	160.95 ± 4.09	171.29	171.29	21.8
L1	5.32 ± 0.66	84.43 ± 4.18	86.33	257.62	11.0
L2	4.63 ± 0.82	64.94 ± 3.83	63.31	320.93	8.1
L3	5.89 ± 0.73	65.16 ± 3.72	69.45	390.38	8.9
Time-to-L4	5.69 ± 0.30	371.66 ± 8.47	390.47	390.38	49.8
L4	4.70 ± 0.01	97.44 ± 7.11	95.45	485.83	12.2
L5	2.40 ± 0.99	211.06 ± 13.45	177.62	663.45	22.6
PP	3.01 ± 1.17	138.01 ± 10.88	120.58	784.03	15.4
Time-to-Pupa	4.61 ± 0.29	804.96 ± 17.30	783.09	784.03	100.0

To simplify the estimation of accumulated degree-days through different life-history stages, it is necessary to assume a common lower developmental threshold for all stages. This approach was used by Manel and Debouzie (1997), who fixed the threshold at 0°C to predict degree-day accumulation through four larval stages of the Chestnut weevil, *Curculio elephas* Gyllenhal. Reference to Figure 3.13 suggests that a lower developmental threshold of 5°C may be suitable for this purpose in *M. privata*. Generally, if the fixed threshold is below the original threshold for a particular stage, the 'new' thermal constant for that stage will increase to compensate for the lowered threshold; whereas if the fixed threshold is above the original threshold, the new thermal constant will decrease to compensate for the raised threshold. Estimated thermal constants for each stage above a fixed threshold of 5°C, determined by regressing development rate against 'temperature minus 5°C' and constraining the regression line to pass through the axes origin, are also presented in Table 3.11. The most notable point is that of the 784 DD required above 5°C to complete egg-larval development, approximately half the time, or 390 DD, is occupied by time-to-L4, while the damaging phase (L4+L5) occupies approximately 35 percent of the egg-larval period. This means that in physiological terms, more time is available to detect eggs and small larvae (L1-L3) than is taken by fourth and fifth instars to cause significant damage.

3.4.4 Experiment 4: Degree-days required for pupal development

Pupal development at 11.5 to 24°C

Pupae developed to adult eclosion at all temperatures from 11.5 to 24°C. Mean development times for each temperature are presented in Table 3.12, while the relationship between temperature and the development rate of individuals is presented in Figure 3.14. Mean (± SE) development time decreased from 77.3 ± 2.6 days (n=13) at 11.5°C to 37.0 ± 2.1 days (n=9) at 17.5°C, but no significant

Table 3.12 The mean number of days required by *M. privata* for adult differentiation at mean temperatures ranging from 11.5 to 24°C.

Mean Temperature	No. Pupae	Mean Duration (days)	SE	Range (days)	No. Adults Emerged	% Survival
11.5	21	77.3	2.6	64 - 92	13	61.9
15.0	18	52.2	1.6	42 - 64	16	88.9
17.5	10	37.0	2.1	27 - 49	9	90.0
20.0	5	43.8	6.2	25 - 61	5	100.0
21.0	15	46.3	3.8	28 - 68	13	86.7
23.8	8	53.7	4.1	40 - 68	6	75.0
24.0	15	38.1	3.3	25 - 56	11	73.3
Total	92	51.2	1.9	25 - 92	73	79.3

Table 3.13 Summary table showing whether or not the mean duration of the period of adult differentiation in *M. privata* pupae differed significantly between temperatures. Probabilities are based on T-tests. * = $p < 0.05$, ** = $p < 0.01$

Temperature		11.5	15.0	17.5	20.0	21.0	23.8	24.0
(°C)	Mean Days	77.3	52.2	37.0	43.8	46.3	53.7	38.1
11.5	77.3		**	**	**	**	**	**
15.0	52.2			**	0.07	0.13	0.68	**
17.5	37.0				0.23	0.07	**	0.79
20.0	43.8					0.73	0.21	0.38
21.0	46.3						0.25	0.12
23.8	53.7							*
24.0	38.1							

reduction in mean development time was achieved at higher temperatures, where mean development times ranged from approximately 38 to 54 days and were highly variable (Table 3.13).

A significant relationship was found between pupal development rate and temperature over the range 11.5 to 24°C ($F_{1,71} = 27.18$, $p<0.001$), but only 28 percent of total variation in pupal development rate was explained by temperature (Fig. 3.14). No significant relationship was found over the range 15 to 24°C

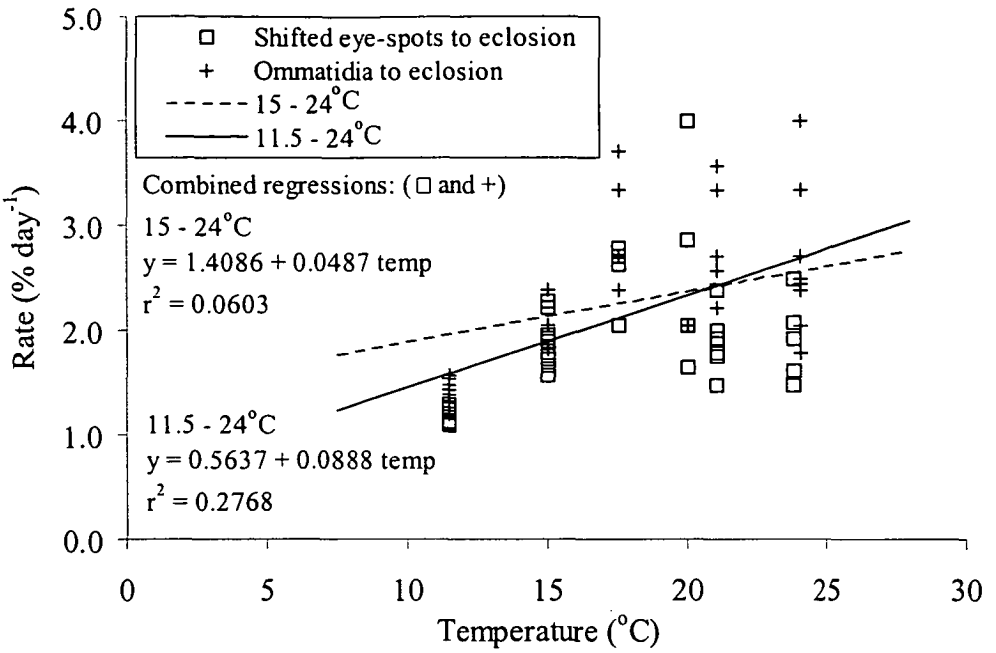


Figure 3.14 The development rate of *M. privata* pupae in relation to temperature. The results are based on 38 pupae transferred at the shifted eye-spots stage and 35 pupae transferred at the developing ommatidia stage. The solid line represents a significant regression over the range 11.5 to 24°C, while the broken line represent a non-significant regression over the range 15 to 24°C.

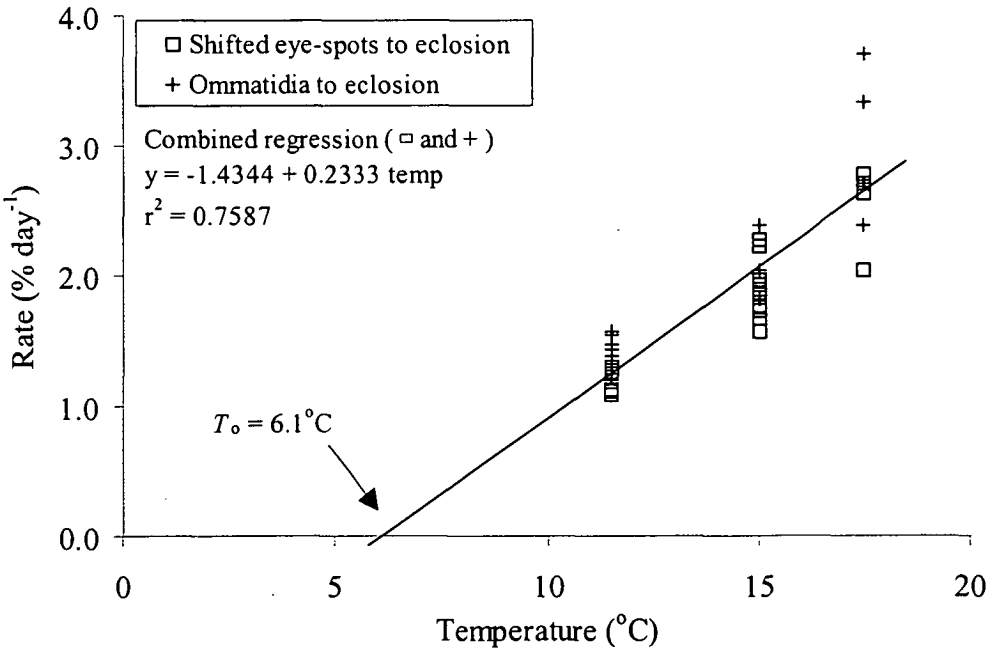


Figure 3.15 The development rate of *M. privata* pupae in relation to temperatures between 11.5 and 17.5°C. The regression line represents pupae requiring 429 DD above a threshold of 6.1°C for adult differentiation.

($F_{1,58} = 3.72$, $p = 0.059$, $r^2 = 0.06$) (Fig. 3.14). These regression results indicate that temperature was a poor predictor of pupal development rates between 11.5 and 24°C, which was in marked contrast to previous results for egg and larval development rates, which could be reliably predicted by temperature over this same range (Fig. 3.13).

Despite the fact that pupal development rate was poorly related to temperature over the range 11.5 to 24°C, reference to Figure 3.14 does suggest a linear response over the range 11.5 to 17.5°C. This hypothesis is supported by the fact that mean development times significantly decreased between 11.5 and 17.5°C (Table 3.13). A strong linear relationship between development rate and temperature was found over this limited temperature range ($F_{1,36} = 113.19$, $p < 0.001$, $r^2 = 0.76$) (Fig. 3.15), from which result the temperature requirements for adult differentiation were estimated at 428.7 ± 40.3 DD > 6.1 ± 0.8°C. Furthermore, after fixing T_0 at 5°C as before to enable degree-days to be accumulated through different life-history stages, the thermal constant estimated for adult differentiation increased to 484 DD. Adding this figure to the 784 DD required for egg-larval development (Table 3.11) provided an estimate of 1268 DD > 5°C as the minimum requirement for *M. privata* to complete a generation (Table 3.14).

Table 3.14 The estimated number of degree-days above 5°C required by *M. privata* to complete an entire generation.

Stage	T_0 (°C)	K (DD)	DD > 5°C
Time-to-Pupa	4.61 ± 0.29	804.96 ± 17.30	784.03
Adult differentiation	6.15 ± 0.80	428.65 ± 40.29	484.50
Generation time			1268.53

3.4.5 Adult differentiation consisted of two distinct stages of development

Adult differentiation could be divided into two distinct stages of development, termed 'stage-1' and 'stage-2' of adult differentiation (Fig. 3.16) on the basis of different temperature responses (Figs. 3.17 and 3.18). Stage-1 of adult differentiation is defined as the period between the onset of development and the unpigmented pharate adult, while stage-2 of adult differentiation is defined as the period between the unpigmented pharate adult and adult eclosion. Development rate increased with temperature during stage-1 ($F_{1,74}$ = 412.5, $p < 0.001$, r^2 = 0.85) (Fig. 3.17), whereas no significant relationship was found between temperature and development rate during stage-2 ($F_{1,63}$ = 0.23, p = 0.63, r^2 = 0.0037) (Fig. 3.18). From these regression results, 289.4 ± 14.2 DD > 5.1 ± 0.7°C were estimated for stage-1 of adult differentiation (290.7 DD > fixed threshold of 5°C) but the temperature requirements for stage-2 could not be determined with any certainty. Subtracting the stage-1 requirement (290.7 DD) from the overall requirement for adult differentiation (484.5 DD from Table 3.14) provided a preliminary estimate of 193.8 DD > 5°C for stage-2. However, as development rates of 2-4% per day at 24°C (see Fig. 3.18) corresponded to 475-950 DD > 5°C (25-50 days at 19 DD per

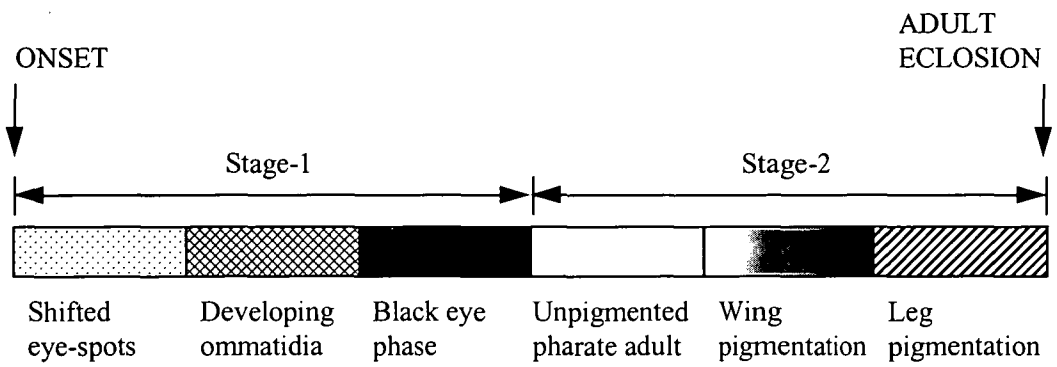


Figure 3.16 Of the six states recognised during adult differentiation, the first three make up 'stage-1' of adult differentiation, which is defined as the period between the onset of adult differentiation and the unpigmented pharate adult, while the last three make up 'stage-2' of adult differentiation, which is defined as the period between the unpigmented pharate adult and adult eclosion.

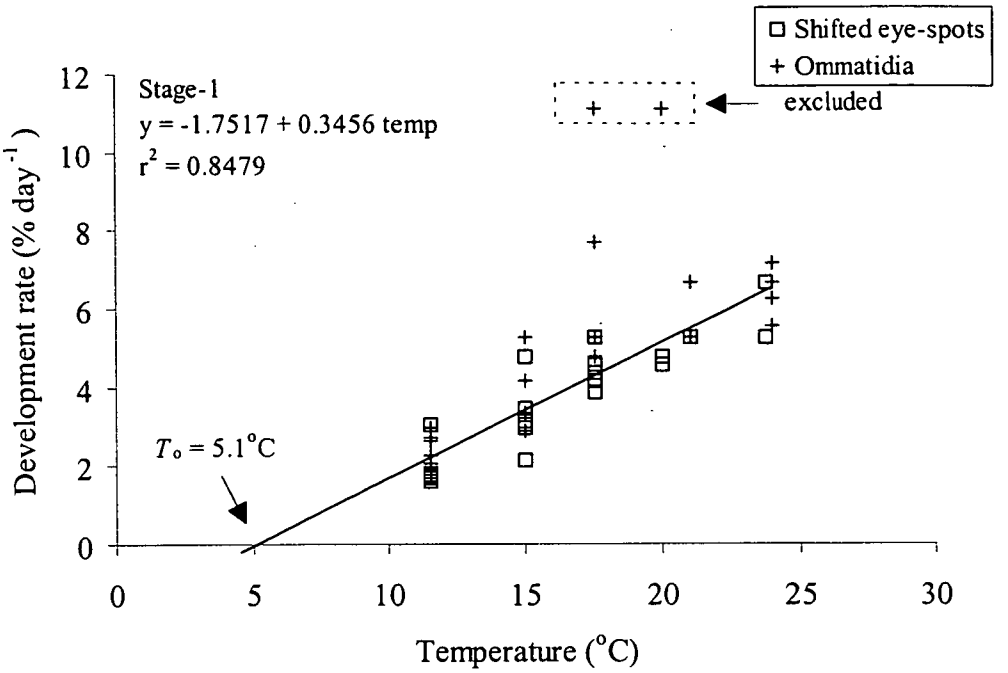


Figure 3.17 The development rate of *M. privata* pupae between the onset of adult differentiation and the unpigmented pharate adult (stage-1 of adult differentiation) in relation to temperature. The two individuals with very high development rates excluded from analysis were assumed to have been near the end of the developing ommatidia state when selected for use in the experiment.

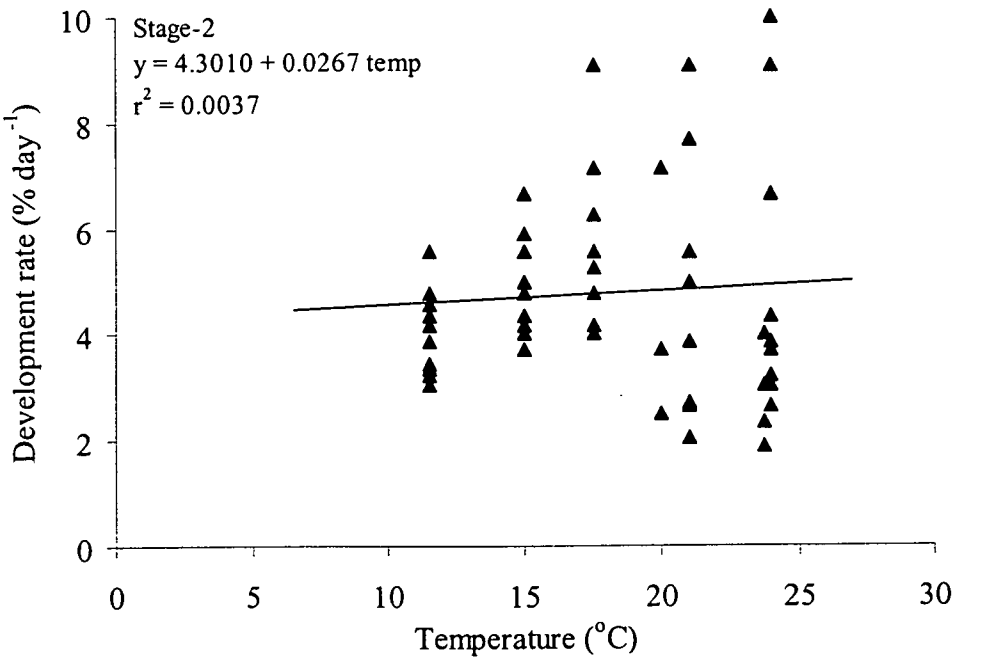


Figure 3.18 The development rate of *M. privata* pupae between the unpigmented pharate adult and adult eclosion (stage-2 of adult differentiation) in relation to temperature.

day), significantly more than 194 DD was often required for stage-2 development at higher temperatures. This means that thermal summation probably cannot be used to predict stage-2 development times at high temperatures.

Different temperature responses during different stages of adult differentiation were first suspected when preliminary results showed that overall development times at 20, 21 and 23.8°C were not significantly different from that at 15°C (Table 3.13). The result was surprising because it was evident from visual examinations of developing pupae that pupae placed at 20-24°C usually developed to an unpigmented pharate adult faster than pupae placed at lower temperatures. For overall development times at 20-23.8°C and at 15°C to remain similar despite more rapid development to the unpigmented pharate adult at higher temperatures, it was hypothesised that development from the unpigmented pharate adult to adult eclosion was inhibited in some way, and hence took longer, at higher temperatures. This hypothesis is shown schematically in Fig. 3.19. The mean number of days taken to complete stage-1 and stage-2 of adult differentiation were determined to test this

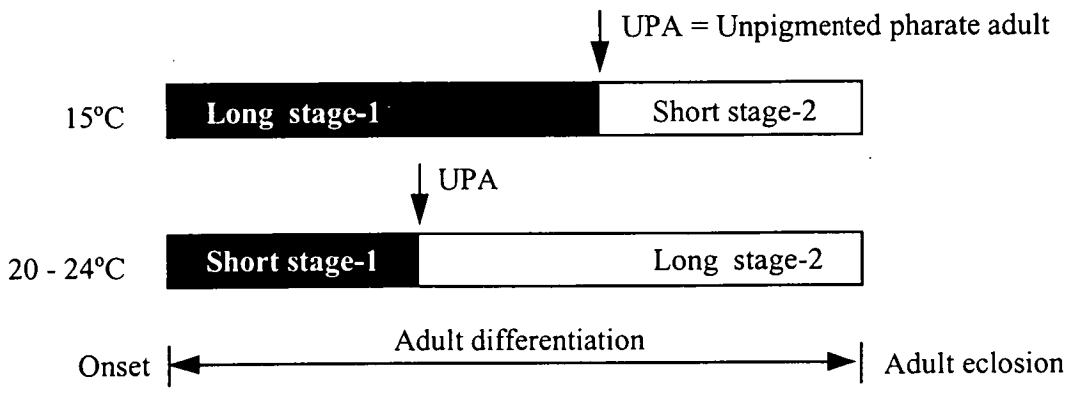


Figure 3.19 Schematic diagram illustrating how a similar period of adult differentiation may be achieved at 20-24°C and at 15°C despite more rapid development to the unpigmented pharate adult at higher temperatures. It was hypothesised that development from the unpigmented pharate adult to adult eclosion was inhibited by high temperatures and therefore took longer than at 15°C.

hypothesis and are presented in Table 3.15. Significance levels for between-temperature comparisons of mean development times for stage-1 and stage-2 are presented in Tables 3.16 and 3.17 respectively. Mean development times for stage-1 tended to decline as temperature increased, ranging from 49.5 ± 2.3 d at 11.5°C (n = 18) to 15.1 ± 0.3 d at 24°C (n = 15) (Table 3.15). However, mean development times between 17.5 and 23.8°C were not significantly different (Table 3.16). Meanwhile, mean development times for stage-2 tended to be longer at 20-24°C than at 15°C (Table 3.15), but only the mean of 36.7 ± 4.0 d (n = 6) at 23.8°C was significantly longer than 20.9 ± 1.1 d (n = 14) at 15°C ($p < 0.01$) (Tables 3.15 and 3.17). The latter result was preliminary evidence of high temperatures inhibition of stage-2 development, but remaining comparisons between 15°C and higher temperatures up to 24°C were not significant (Table 3.17).

Further breakdown of stage-2 development revealed that the time spent as an unpigmented pharate adult in particular was highly variable at 20-24°C, ranging from 5 to 35 days and exceeding 14 days in 11 out of 20 individuals (Fig. 3.20a). In contrast, the time spent as an unpigmented pharate adult at 15-17.5°C ranged from 5 to a maximum of just 13 days (n = 12) (Fig. 3.20a). Once wing pigmentation began, development continued uninterrupted to adult eclosion and tended to decline in duration with increasing temperature ($F_{1,41} = 40.79$, $p < 0.001$, $r^2 = 0.50$) (Fig. 3.20b). Figure 3.20a suggests that 7-14 days should be sufficient time for unpigmented pharate adults to develop to the next state (wing pigmentation) at 20-24°C. Thus, the 11 pupae remaining as unpigmented pharate adults for more than 14 days at ≥ 20°C were considered to have spent the additional time in a state of high-temperature or summer dormancy, otherwise known as 'aestivation' (see Pedigo (1989), p. 163).

Table 3.15 The mean duration (days) and proportion of pupal development time spent in Stage-1 and Stage-2 of adult differentiation by *M. privata*.

Mean Daily Temperature	Duration of Stage-1				Duration of Stage-2				Proportion in each stage (%)
(°C)	Mean	SE	n	Range	Mean	SE	n	Range	Stage-1 : Stage-2
11.5	49.5	2.3	18	33-63	26.0	1.4	12	18-33	66 : 34
15.0	30.9	1.8	14	19-47	20.9	1.1	14	15-27	60 : 40
17.5	19.7	1.6	10	9-26	18.0	1.6	9	11-25	52 : 48
20.0	17.3	4.2	3	9-22	27.0	7.5	3	14-40	39 : 61
21.0	18.6	0.4	11	15-19	27.2	4.7	10	11-49	41 : 59
23.8	16.7	0.8	7	15-19	36.7	4.0	6	25-53	31 : 69
24.0	15.1	0.3	15	14-18	22.7	3.0	11	10-38	40 : 60

Table 3.16 Summary table showing whether or not the mean duration of stage-1 of adult differentiation in *M. privata* pupae differed significantly between temperatures. Probabilities are based on T-tests. * = $p < 0.05$, ** = $p < 0.01$

Temperature		11.5	15.0	17.5	20.0	21.0	23.8	24.0
(°C)	Mean days	49.5	30.9	19.7	17.3	18.6	16.7	15.1
11.5	49.5		**	**	**	**	**	**
15.0	30.9			**	**	**	**	**
17.5	19.7				0.53	0.51	0.17	**
20.0	17.3					0.54	0.83	0.24
21.0	18.6						*	**
23.8	16.7							*
24.0	15.1							

Table 3.17 Summary table showing whether or not the mean duration of stage-2 of adult differentiation in *M. privata* pupae differed significantly between temperatures. Probabilities are based on T-tests. * = $p < 0.05$, ** = $p < 0.01$

Temperature		11.5	15.0	17.5	20.0	21.0	23.8	24.0
(°C)	Mean days	26.0	20.9	18.0	27.0	27.2	36.7	22.7
11.5	26.0		**	**	0.82	0.80	**	0.31
15.0	20.9			0.13	0.14	0.15	**	0.54
17.5	18.0				0.09	0.10	**	0.20
20.0	27.0					0.98	0.25	0.54
21.0	27.2						0.19	0.43
23.8	36.7							*
24.0	22.7							

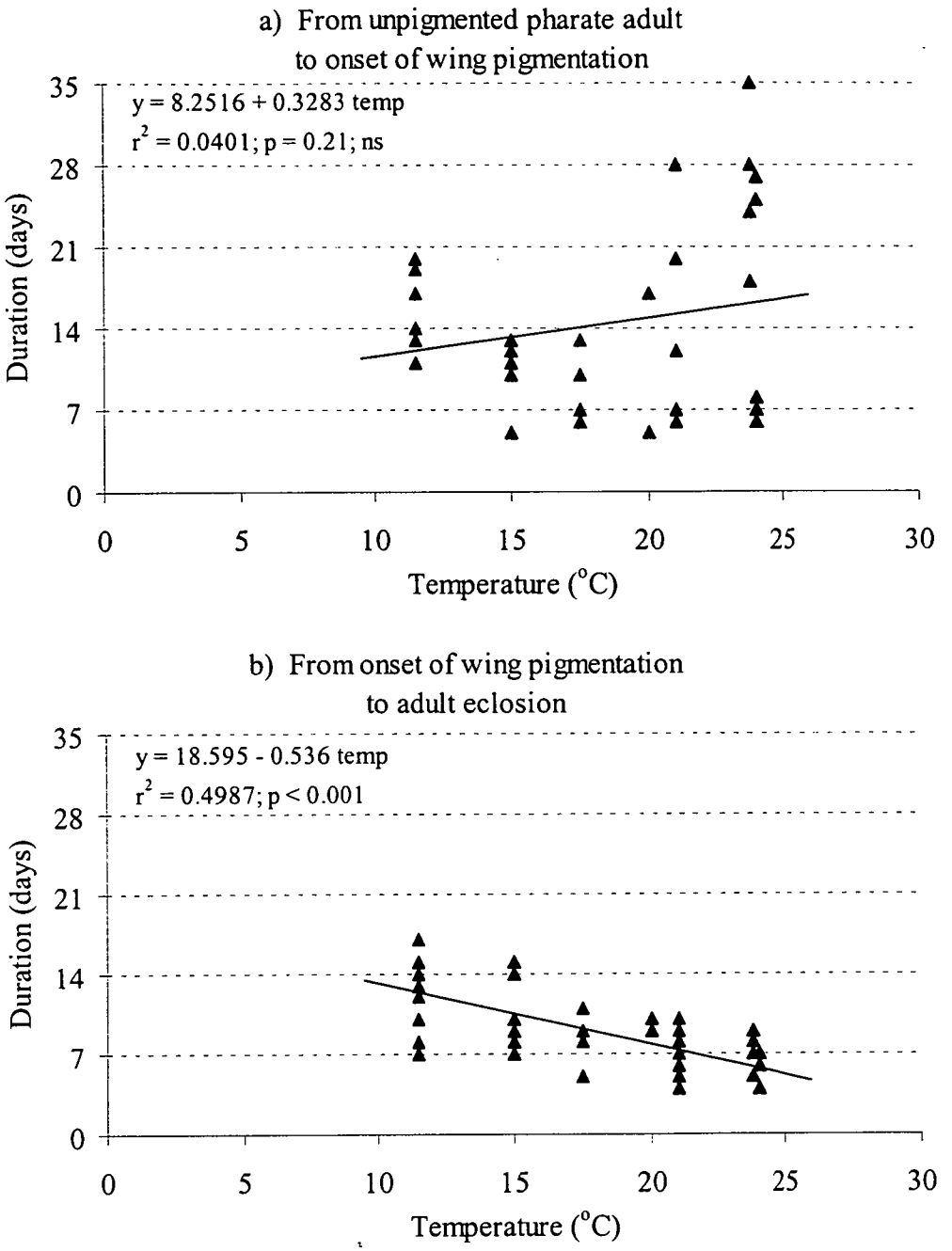


Figure 3.20 The number of days (a) spent as an unpigmented pharate adult, and (b) from the onset of wing pigmentation to adult eclosion during stage-2 of adult differentiation in *M. privata* pupae. Only the regression in (b) is significant.

Effect of high temperatures on pupal development

At this point, it is timely to consider the effect of the additional high temperatures (26-35°C) on pupal development of *M. privata*. The effects of the additional high temperature treatments on stage-1 and stage-2 of adult differentiation are presented in Table 3.18.

- Fourteen pupae placed at 26°C completed stage-1 of adult differentiation in about ten days (Table 3.18). Thereafter, three pupae remaining at 26°C completed stage-2 of adult differentiation with a mean duration of 34.7 ± 3.0 days, significantly longer than the mean duration of 23.7 ± 0.9 d (n = 6) required at 15°C (Table 3.18).
- Thirteen pupae placed at 30°C also completed stage-1 of adult differentiation in about ten days (Table 3.18). However, no pupae remaining at 30°C completed stage-2 of adult differentiation, whereas five pupae transferred to 15°C at the end of stage-1 completed stage-2 with a mean duration of 23.8 ± 1.5 d (Table 3.18).
- Fourteen pupae placed at 35°C died within seven days while still at the earliest stages of adult differentiation (shifted eye-spots or developing ommatidia). Dead pupae did not writhe their abdomen when prodded with a blunt instrument and turned dark brown within about a week.

As the mean durations for stage-2 development at 26°C and at 23.8°C were both significantly longer than mean durations at 15°C, together they provide limited evidence supporting the hypothesis that development of the pharate adult is inhibited by high temperatures. Also, the fact that no adults emerged from pupae remaining at 30°C belies the fact that three unpigmented pharate adults survived (abdomens writhed when pupae were prodded) for 60 days at 30°C without further development (see footnotes for Table 3.18). This was a key finding as it further indicated that

Table 3.18 The effect of high temperatures on the duration of stage-1 and stage-2 of adult differentiation during the pupal stage of *M. privata*.

Treat-ment	No. Pupae	Stage-1				Stage-2				Overall			
		Temp. °C	Mean ± SE	n	Range	Temp. °C	Mean ± SE	n	Range	Mean ± SE	n	Range	% Survival
1	7	26	9.7 ± 0.9 a	7	7 - 12	26	34.7 ± 3.0 a	3	29 - 39	44.7 ± 3.3 a	3	38 - 48	43
2	7	26	9.7 ± 0.9 a	7	7 - 12	15	23.7 ± 0.9 b	6	21 - 27	33.0 ± 1.2 b	6	28 - 36	86
3	7	30	10.0 ± 0.9 a	7	7 - 12	30	-	0[Ψ]	-	-	-	-	0
4	6	30	9.7 ± 1.1 a	6	7 - 14	15	23.8 ± 1.5 b	5	19 - 28	34.0 ± 2.7 b	5	26 - 40	83
5	7	35	-	0	-	35	-	0	-	-	0	-	0
6	7	35	-	0	-	15	-	0	-	-	0	-	0

Notes: Within each column of means, means followed by different letters are significantly different, based on T-tests ($p<0.05$).

Ψ Although no adults emerged when pupae remained at 30°C, three of the seven unpigmented pharate adults survived for 60 days without further development, after which they were transferred to 15°C. At 15°C, one individual died before developing further, while two developed wing and leg pigmentation but died before emergence.

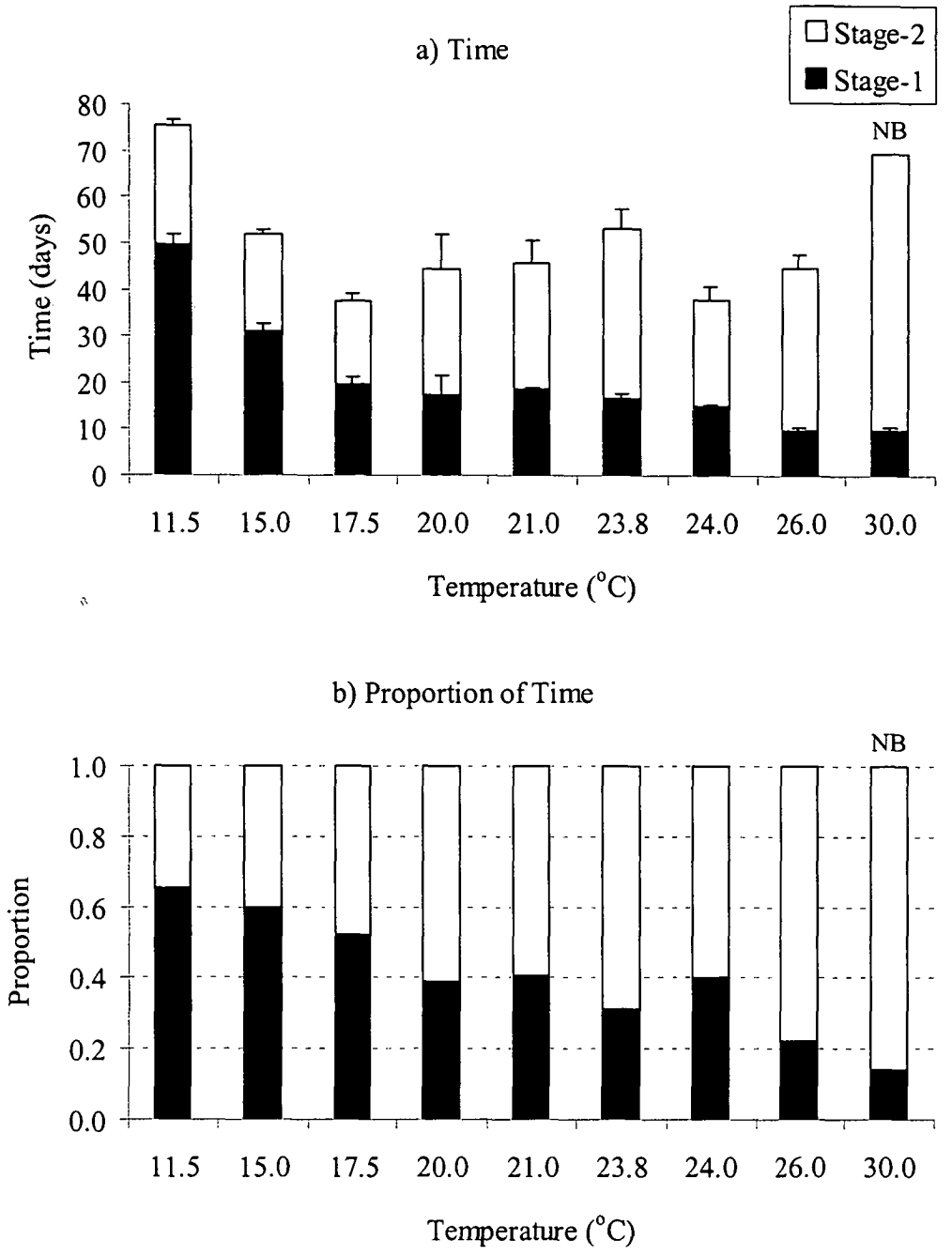


Figure 3.21 The mean number of days and proportion of total development time spent in stage-1 and stage-2 of adult differentiation in relation to temperature. Error bars in (a) refer to one SE. Proportions in (b) are based on the means in (a). NB: Although no adults emerged at 30°C, three unpigmented pharate adults survived for 60 days at that temperature without further development. Hence, the 70 days for 30°C in (a) indicates how long pupae could survive at 30°C, not how long it took to complete development. The final column in (b) indicates that of the 70 days survived at 30°C, just 14% of the time was required to complete stage-1 of adult differentiation. The remaining time was occupied by aestivating unpigmented pharate adults.

pupae have the capacity to develop partially, then aestivate as pharate adults in direct response to high temperatures. The possibility that pharate adults can aestivate for up to two months at high temperatures has major implications for *M. privata* adult phenology and will be considered further in the discussion.

Finally, the results from pupal development studies at all temperatures are summarised in Fig. 3.21. The figure shows the time and proportion of overall time spent in each of the two stages of adult differentiation at the various temperatures. The most notable points are that: (i) stage-1 duration declined as temperature increased; (ii) stage-2 durations at 20°C and above were similar to or longer than at lower temperatures; and (iii) the *proportion* of time spent in stage-2 tended to increase with temperature.

3.5 DISCUSSION

3.5.1 Temperature requirements, climate and M. privata *phenology*

At the start of this thesis (Section 1.4), it was hypothesised that the contrasting phenologies of *M. privata* in Tasmania were alternative life-history strategies in response to different climatic environments. Hence, it is necessary for this thesis to demonstrate that: (i) localities with contrasting phenologies have different climatic environments; and (ii) different climatic environments cause the different phenologies. The first matter is solved directly by comparing the climates of two summer emergence sites in Tasmania (*Surrey Hills* and Tarraleah) with several areas in southern Australia where *M. privata* adults emerge from pupation in autumn.

Tasmania is a relatively mountainous island with latitudinal and altitudinal ranges of 40°38' to 43°39' south and 0 to 1617 m ASL respectively (Australian Bureau of Meteorology, 1995). The climate is classified as temperate maritime, since no point in Tasmania is further than 115 km from the coastline. However, local

temperatures can vary greatly because of distance from the coast and elevation in particular, since temperature decreases by an average 0.7°C per 100 m increase in elevation. Hence, *Surrey Hills* and Tarraleah, both situated at around 600 m ASL, tend to be about 4-5°C cooler than Hobart throughout the year (Fig. 3.22). Furthermore, when compared with other areas of *M. privata*'s distribution, summer emergence sites stand out as being relatively mild during summer, with just 1-2 days annually when the maximum temperature exceeds 30°C, and particularly cold during winter, with over 110 days annually when the minimum temperature falls below 2°C (Table 3.19). Elevated inland areas of Tasmania also experience widespread severe frosts (< 0°C) above 300 m ASL and snowfalls above 900 m ASL (Australian Bureau of Meteorology, 1995). Thus, sites with contrasting phenologies have different climatic environments. Having established that localities with contrasting phenologies have different climatic environments, the next requirement is to show how different climatic environments in Tasmania might cause different phenologies of *M. privata*. The results of this chapter address this latter requirement.

3.5.2 Temperature requirements for egg-larval development

The primary objective of this chapter was to determine the temperature requirements (T_0 and K) for *M. privata* development so that it would be possible to predict development times in the field from local average temperatures. As development rates for eggs and larvae were almost linearly related to temperature over the range 11.5-24°C (Fig. 3.13), lower developmental thresholds and thermal constants for these stages could be determined readily by regression analysis (Table 3.11). Factors such as geographic origin, intra-population variation (i.e. family effects) and laying order were found to be of little practical importance in this study.

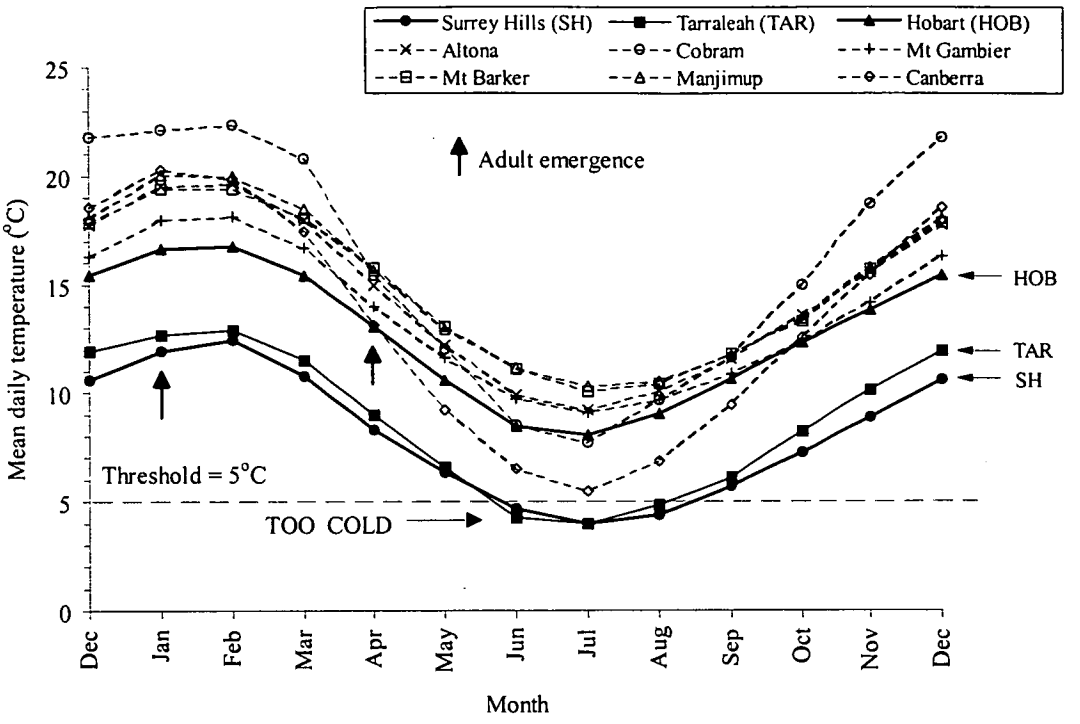


Figure 3.22 Mean daily temperatures for nine localities where *M. privata* is found. Solid lines refer to Tasmanian localities, broken lines to localities on mainland Australia. The vertical arrow for January refers to peak adult emergence during mid-summer at elevated localities in Tasmania, such as *Surrey Hills* and Tarraleah. The vertical arrow for April refers to peak adult emergence during mid-autumn elsewhere. Field temperatures during egg-larval development typically average about 12°C or lower, regardless of locality. Note the temperature difference of about 4°C between Hobart (HOB) and the two elevated localities in Tasmania (SH and TAR) throughout the year. (Source: Australian Bureau of Meteorology, 1998).

Table 3.19 The mean annual number of days exceeding 30°C and below 2°C (light frost) for several localities in Tasmania and mainland Australia where *M. privata* is distributed. (Source: Australian Bureau of Meteorology, 1998).

Locality		Elevation (m ASL)	Annual No. Days Maximum Temperature ≥ 30°C	Annual No. days Minimum Temperature ≤ 2°C.
Tasmania				
Surrey Hills	(Waratah)	612	1.0	113.4
Tarraleah		589	2.2	142.0
Hobart		50	5.8	16.4
Mainland				
Altona	Vic.	18	25.4	18.5
Cobram	Vic.	110	53.4	35.1
Mt. Gambier	S.A.	63	24.1	23.0
Mt. Barker	W.A.	280	29.8	3.6
Manjimup	W.A.	280	31.2	3.2
Canberra [a]	A.C.T.	578	29.2	98.6

[a] The city of Canberra is unusually cold during winter compared to other mainland localities where *M. privata* is found because its elevation of 578m ASL makes it prone to frequent frosts.

Considering the developmental threshold first, T_0 for particular life-history stages ranged from around 5.9°C for eggs and L3 to 2.4°C for L5 (Table 3.11). These thresholds are lower than the predicted values of 7.7°C to 11.9°C made at the start of this chapter using mean daily temperatures at certain periods in the life-cycle as simple estimates (Gilbert, 1988; Gilbert & Raworth, 1996). Therefore, whilst such simple methods may be useful to show general relationships between developmental thresholds and average field temperatures, as in Gilbert's (1988) study, they should not be relied on if precision is required, e.g. for detailed phenology models. Campbell *et al.* (1974) suggested that lower developmental thresholds for late-instar larvae of *Pieris rapae* ensure that declining temperatures do not prevent completion of the generation before winter. In comparison, the low developmental threshold of 2.4°C for fifth-instar *M. privata* larvae would enable larval development to continue during winter in all but the coldest areas (see below).

Despite the fact that egg and larval stages have different developmental thresholds (Table 3.11), for practical purposes a threshold of 5°C can probably be assumed for the entire egg-larval period (but see below). This is suggested because: (a) a threshold of 4.6°C was found for the overall period between oviposition and pupation (Table 3.11), and (b) egg and larval development rates usually doubled between 10°C and 15°C and almost doubled again between 15°C and 24°C (Fig. 3.13). Doubling of development rates over these two temperature ranges reflects that roughly twice as many degree-days above 5°C are accumulated at 15°C than at 10°C, and also at 24°C than at 15°C. Even though 5°C is about twice as high as the 2.4°C threshold estimated for L5, a threshold of 5°C would still permit development during winter in most areas of *M. privata*'s distribution as mean daily temperatures during winter usually remain above 5°C (Fig. 3.22). However, at

Tasmania (e.g. *Surrey Hills* and Tarraleah), mean daily temperatures during winter are usually below 5°C (Fig. 3.22) and may thus prevent development. Therefore, low winter temperatures (near or below *M. privata*'s developmental threshold) in elevated areas could be a major reason why seasonal activity of *M. privata* begins in mid-summer at high altitudes in Tasmania. Commencing seasonal activity in mid-summer would be advantageous as it would allow larval development to be completed before winter. A later start to seasonal activity, e.g. in autumn as elsewhere, could be adverse to development and survival and therefore limit reproductive potential. The development of many insect species occurring in elevated localities of south-eastern Australia is similarly crowded into the warmer months because these areas experience the most extreme winter conditions in Australia (Norris, 1991).

Whilst initiating seasonal activity during mid-summer may be advantageous at higher altitudes in Tasmania, the same behaviour elsewhere, particularly on mainland Australia, may be hazardous to survival. During summer, temperatures above 30°C are commonly experienced on mainland Australia but are only occasionally experienced in Tasmania (Table 3.19). However, very high temperatures (>35°C) may occur at low altitudes in Tasmania, particularly in the east and south-east of the state, where Hobart and nearby Bushy Park share the state's highest recorded temperature of 40.8°C (Australian Bureau of Meteorology, 1995). Experiments 1 and 2 found that *M. privata* eggs died when incubated at 30°C (Fig. 3.10). Therefore, an autumn start to the life-cycle in warmer regions would be advantageous as egg development would coincide with milder autumn conditions.

It is interesting to note that mean daily temperatures at the two summer emergence sites in Tasmania are about 4°C cooler than at Hobart throughout the year

(Fig. 3.22). This temperature difference is towards the upper end of the predicted global average temperature change of 2-5°C over the next fifty years due to the greenhouse effect (Schneider, 1992). Since the temperature difference between these sites is currently associated with contrasting phenologies, perhaps *M. privata* adult phenology at high altitudes in Tasmania will shift from summer to autumn in direct response to the anticipated global warming, thereby coming into line with the phenology presently found elsewhere. Alternatively, phenological differences in Tasmania could be maintained if lowland populations emerge later in autumn and winter.

As development rates of eggs and larvae are strictly temperature-related, thermal summation can probably be used to predict development times of these stages in the field. For instance, one useful application could be to predict the time available for population control after detecting a large egg population. Since *M. privata* larvae cause over 96% of feeding damage during the final two instars (L4-L5) (Phillips, 1996), the period from peak oviposition to the start of the fourth instar (time-to-L4) is very important as it represents the developmental time before the onset of significant defoliation. Consequently, control of *M. privata* is recommended during this period (e.g. Neumann and Collett, 1997). As noted in the results, approximately 390 DD or half the egg-larval period is occupied by non-damaging early stages (eggs to L3) (Table 3.11). Therefore, assuming a lower developmental threshold of 5°C and mean daily temperatures of around 12°C, typical of field temperatures experienced by *M. privata* during egg-larval development (Fig. 3.22), time-to-L4 would be approximately 56 days (Table 3.20). Hence, the population would need to be controlled within about two months of peak oviposition in order to minimise damage to host trees. Less time would be available for population control at higher temperatures (Table 3.20).

Table 3.20 The predicted durations (in days) of key stages of *M. privata* development at various temperatures between 10 and 25°C.

Stage	Thermal constant (DD > 5°C)	Mean Temperature in °C (and DD > 5°C per day)				
		10 (5)	**12 (7)**	15 (10)	20 (15)	25 (20)
		Predicted Duration (Days)				
Time-to-L4	390	78	**56**	39	26	20
Time-to-pupa	784	157	**112**	78	52	39
Generation time	1268	254	**181**	127	85	63

So far, the developmental times presented in this chapter have all been produced under controlled conditions in the laboratory. At the very end of the introduction to this chapter (Section 3.1), it was noted that phenology models should be field tested to determine how useful laboratory results are in a field situation. With this in mind, preliminary field tests were carried out in which the developmental times of several groups of *M. privata* eggs and larvae were recorded outdoors in Hobart and subsequently related to daily meteorological records from the Hobart Bureau of Meteorology. The trials yielded mixed results (see Appendix B).

- In the first trial (Trial-1 of Appendix B), eggs hatched on the predicted day and took 165.80 DD > 5.87°C, which was 3.0% more that the predicted value of 160.95 DD from Table 3.11. However, if a fixed threshold of 5°C was used as suggested above, eggs hatched after 188.45 DD, which was 10.0% more than the predicted value of 171.29 DD. In the latter case, eggs hatched three days later than predicted.
- In the second trial (Trial-2 of Appendix B), eggs took 6.6% and 19.2% more DD than predicted using lower thresholds of 5.87°C and 5.0°C respectively. This equated to eggs hatching one and six days later than predicted respectively. Also in the second trial, it took 850.85 DD > 5°C to reach the pre-pupal stage, which was 24 days later and 28.2% more DD than predicted using a fixed threshold of 5°C.

- In the third trial, (Trial-3 of Appendix B), eggs took 19.5% and 24.8% more DD than predicted using lower thresholds of 5.87°C and 5.0°C respectively. This equated to eggs hatching four and six days later than predicted respectively. Meanwhile, time-to-L4 took 18.3% more DD and 14 days longer than predicted using a fixed threshold of 5.0°C. However, to finish on a positive note, the mid-point of pupation in the third trial occurred after approximately 786.8 DD > 5.0°C, which was less than 1% more DD than predicted.

In general, outdoor development times were longer than predicted on the basis of laboratory results, with thermal requirements sometimes being 25-30% more than predicted. The use of dataloggers to record actual temperatures experienced by developing eggs and larvae may have yielded better results than the meteorological records used. It appears that using 5°C as a fixed threshold for *M. privata* may not yield good predictions for any one stage in particular, but that predictions may improve as the number of developmental stages passed increases. It is imperative that the potential for thermal requirements to vary from those estimated in this chapter be taken into account before they are used to predict development times in the field.

Although useful for estimating the temperature requirements for insect development, the linear relationships found between egg-larval development rates and temperature over the range 11.5-24°C were unremarkable as the development rate of insects usually increases linearly with temperature over the normal range of temperatures experienced in the field (Campbell *et al.*, 1974; Gilbert, 1988; Gilbert & Raworth, 1996). However, in contrast to egg and larval development rates, pupal development rates were not linearly related to temperature between 11.5 and 24°C

(Fig. 3.14). Not only is this result remarkable, it also has important implications for predicting the phenology of adult *M. privata*, as discussed in the following sections.

3.5.3 Temperature requirements for pupal development

The effect of temperature on pupal development was more complicated than originally anticipated. In Chapter 2, the bulk of pupae were held at either 11.5°C or 15°C and the period of adult differentiation declined from 86.3 ± 1.0 days at 11.5°C to 54.3 ± 0.7 days at 15°C (Section 2.3.1). Consequently, it was anticipated that the duration of the period of adult differentiation would continue to decline as temperature increased. However, the pupal development results in this chapter proved otherwise.

The relationship between pupal development rate and temperature was linear over the range 11.5°C to 17.5°C (Fig. 3.15), but non-linear over the range 11.5°C to 24°C (Fig. 3.14). Several individuals developed rapidly at temperatures above 15°C, but mean development rates at 20-24°C were not significantly higher than at 17.5°C, hence the non-linear relationship overall. Despite the poor overall relationship, the linear range of temperatures could be used to estimate the temperature requirements for pupal development, which were 429 DD > 6.1°C (Table 3.14).

The results for the pupal stage suggest that thermal summation might be useful for predicting adult phenology at lower temperatures (≤ 18°C), but probably cannot be used to predict adult phenology at higher temperatures (>18°C). However, since development rates were usually between 1.5 and 3.0% per day between 15°C and 26°C, perhaps 50 days can be used as an approximate development period for adult differentiation irrespective of temperature if soil temperatures remain between 15°C and 26°C.

The non-linear temperature response of *M. privata* pupae contrasts the linear or shallow sigmoidal temperature response curves reported for pupae of some other lepidopteran species, e.g. *P. rapae* (Gilbert, 1988) and *L. dispar* (Casagrande *et al.*, 1987), but is remarkably similar to the non-linear temperature response curve reported previously for pupae of another geometrid, the autumnal moth *E. autumnata* (Peterson & Nilssen, 1996). Pupal development rates of both geometrids tend to be lowest at low temperatures, increase to a maximum at intermediate temperatures, then remain steady or decline at higher temperatures. Peterson and Nilssen (1996) proposed that delayed emergence of autumnal moth adults (i.e. slower pupal development) at higher temperatures was caused by over-optimal temperatures during the pupal stage. The same conclusion was drawn for *M. privata* in this study, although as the following discussion shows, the effects of higher temperatures on pupal development changed as pupae developed.

3.5.4 High temperatures accelerate early pupal development but inhibit late pupal development

A key finding in this chapter was that the effect of temperature on pupal development changed as pupae developed. Once pupae initiated development, the initial response was to develop faster as temperature increased. Consequently, the time taken by pupae to develop to unpigmented pharate adults declined from about 50 days at 11.5°C to just 10 days at 26-30°C (Fig. 3.21a). However, once pupae reached the unpigmented pharate adult state, the temperature response changed and higher temperatures did not necessarily lead to faster adult emergence. On the contrary, although some individuals completed stage-2 of adult differentiation rapidly at high temperatures, many individuals took longer to complete stage-2 at 20-26°C than at 15°C (Fig. 3.23). Development rates were also more variable at higher temperatures than at lower temperatures (Fig. 3.23).

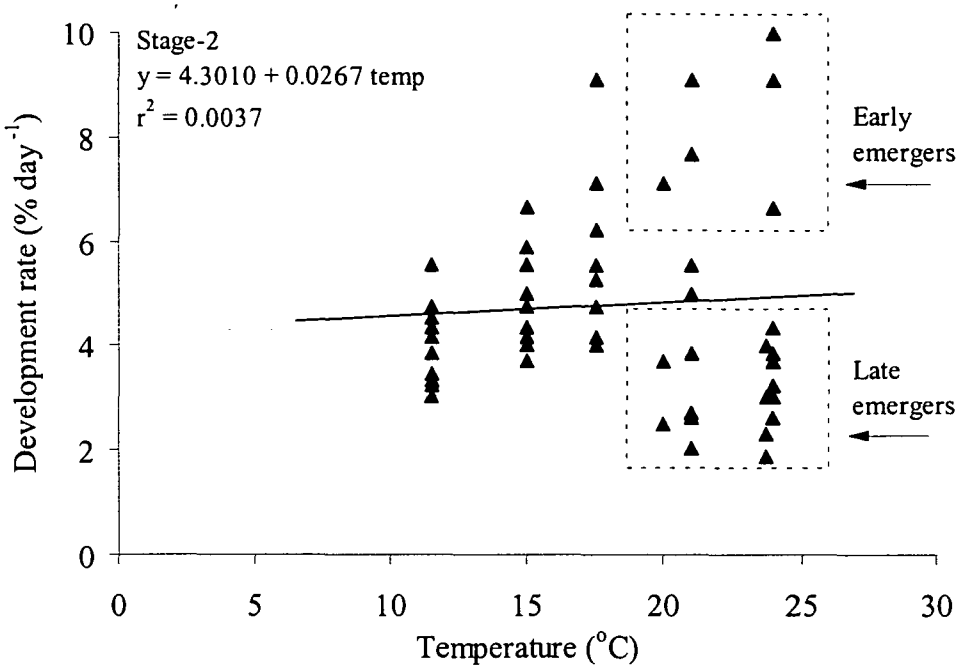


Figure 3.23 The development rate of *M. privata* pupae during stage-2 of adult differentiation. Variability at 11.5°C to 17.5°C is continuous, whereas variability at higher temperatures tends to be discontinuous.

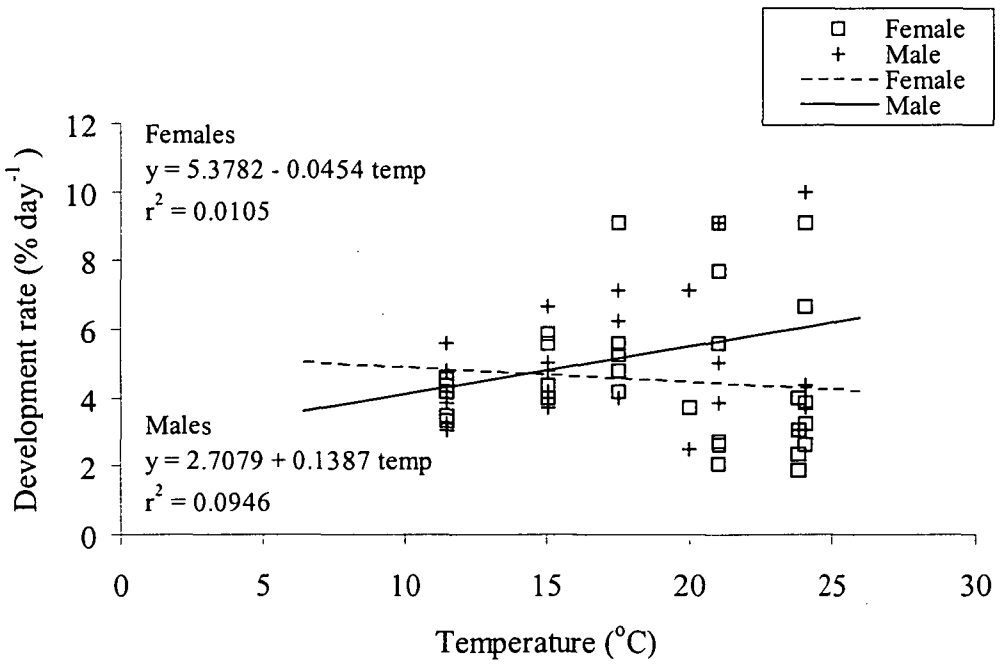


Figure 3.24 The development rate of *M. privata* pupae during stage-2 of adult differentiation in relation to temperature and sex. Regression lines for the different sexes were not significantly different ($p > 0.05$).

Although the underlying reasons for the slower development and increased variability in stage-2 development rates remain uncertain, general patterns emerged from the data and suggested possible causes. First and foremost, although development rates during stage-2 were highly variable at each temperature, the pattern of variability changed with temperature. At 11.5, 15 and 17.5°C, variability was 'continuous' as there was no separation of individuals into discrete groups, whereas at higher temperatures (20-24°C), variability appeared to be 'discontinuous' with some separation of individuals into at least two groups: individuals that developed rapidly and emerged early, and individuals that developed slowly and emerged late (Fig. 3.23). However, the discontinuous variability at higher temperatures may simply reflect a low number of individuals reared. Had more individuals been reared at higher temperatures, perhaps the variability in development rates would also have been continuous.

The variability in stage-2 development rates could not be attributed to pupal sex as regression slopes ($F_{1,61} = 2.72$, $p = 0.104$) and intercepts ($F_{1,62} = 1.66$, $p = 0.202$) for males and females were not significantly different and both sexes were represented evenly across the spectrum of development rates at each temperature (Fig. 3.24). Similar investigations found that pupal weight and geographic origin could also not explain the variation (data not shown). The most plausible explanation found for the discontinuous variation in stage-2 development rates at higher temperatures was that development of the pharate adult appeared to be inhibited to varying degrees by high temperatures. Some individuals developed rapidly (7-10% day^{-1}) and therefore appeared to be unaffected by high temperatures; some individuals developed at intermediate rates (5-7% day^{-1}) and therefore appeared to be moderately affected by high temperatures; while others developed slowly (2-5% day^{-1}) and therefore appeared to be greatly affected by high temperatures

(Fig. 3.23). It is considered that slower development rates during stage-2 at higher temperatures may reflect a period of aestivation (high-temperature dormancy) by the unpigmented pharate adult, as shown schematically in Fig. 3.25. Henceforth for simplicity, this aestivation response of *M. privata* will be referred to as 'late-pupal' aestivation, as it occurs close to the end of the pupal stage.

Previous studies have reported aestival dormancy extending the duration of non-diapause pupal development of other lepidopteran species which, like *M. privata*, live in regions with hot and dry summers. For example, non-diapause pupal development of the cabbage moth, *Mamestra brassicae*, at 20°C may be as short as 20-30 days, but a variable period of aestival dormancy can extend the pupal development period to 35-80 days at the same temperature (Grüner & Sauer, 1988). Grüner and Sauer (1988) suggested that a strong genetical component was involved in variability of the duration of aestivation, which reflected "the frequency of drought periods of a certain length in the past". Those authors considered variability in the duration of aestival dormancy to be of adaptive benefit to *M. brassicae*, as it enabled populations to survive drought periods of unpredictable length. Since *M. privata* experiences hot and dry summers in many areas of its distribution, variability in the duration of late-pupal aestivation may be of adaptive benefit to *M. privata* for the very same reason.

The fact that stage-1 and stage-2 of adult differentiation in *M. privata* pupae exhibit different temperature responses illustrates that not only may the temperature response of a species change from one life-history stage to another, it may also change *during* a particular life-history stage. A similar phenomenon has been reported to occur during the pupal stage of the cabbage root fly, *Delia radicum* (L.) (Diptera: Antomyiidae) (Johnsen *et al.*, 1997). Three distinct phases of development,

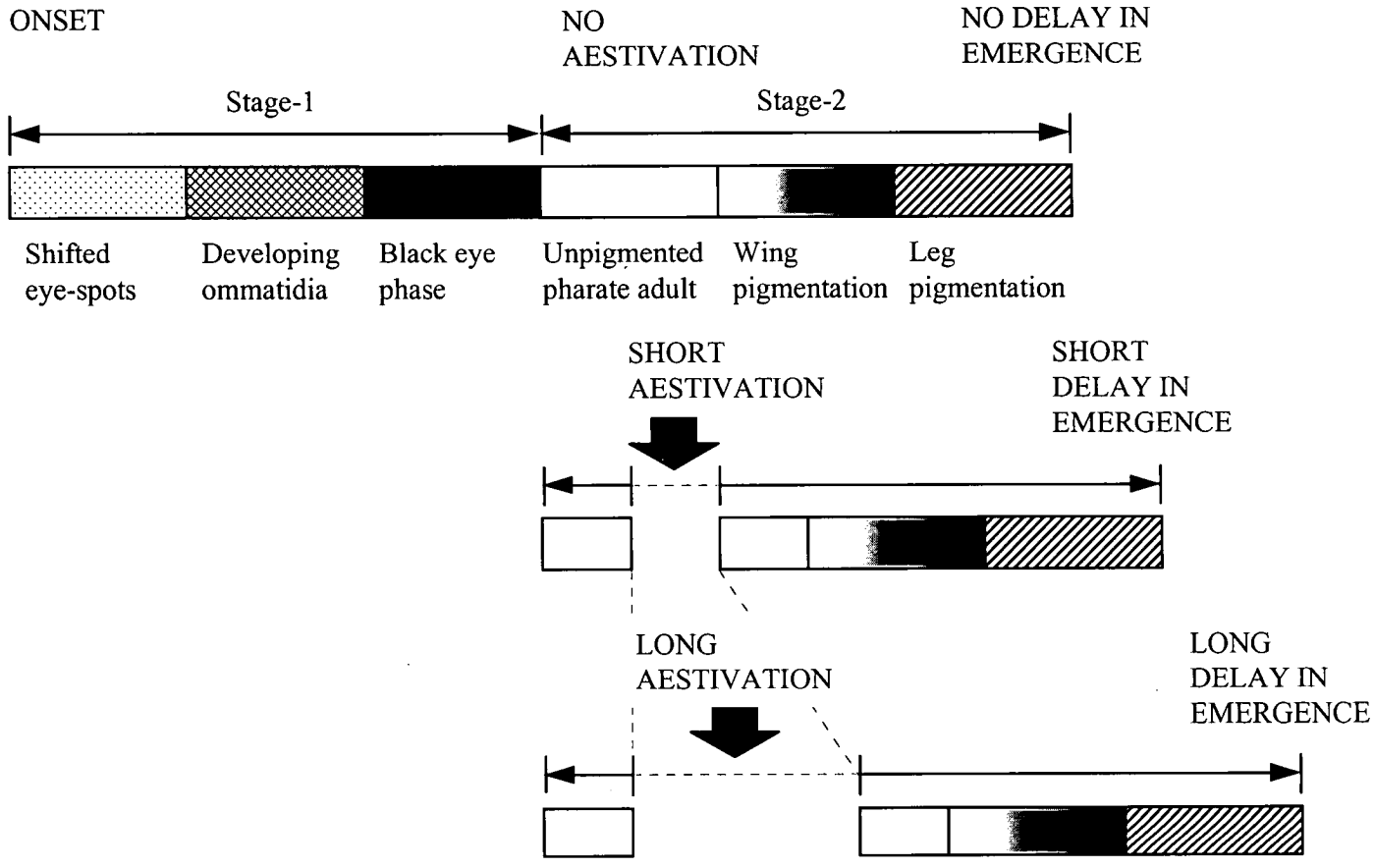


Figure 3.25 Schematic diagram representing how a period of aestivation by the unpigmented pharate adult early in stage-2 of adult differentiation may delay adult emergence. Top: no aestivation leads to adult emergence at the earliest opportunity. Middle: a short aestivation leads to a short delay in adult emergence. Bottom: a long aestivation leads to a long delay in adult emergence.

each with a different response to temperature and a different optima, were recognised during the pupal stage of *D. radicum* (Johnson *et al.*, 1997). Those authors hypothesised that each phase of development was governed by a different enzyme system with a different threshold and response to temperature. Perhaps the two stages of adult differentiation of *M. privata* are also governed by different enzyme systems with different temperature requirements.

3.5.5 Implications for M. privata *phenology*

Assuming that pupae end diapause and begin developing in summer (which seems likely given that adults usually emerge in autumn), pupae probably take about 20-30 days to develop to unpigmented pharate adults, since soil temperatures in late summer and early autumn are typically above 15°C (Fig. 3.26). Once stage-1 of adult differentiation is completed, low temperatures (≤18°C) seem likely to promote

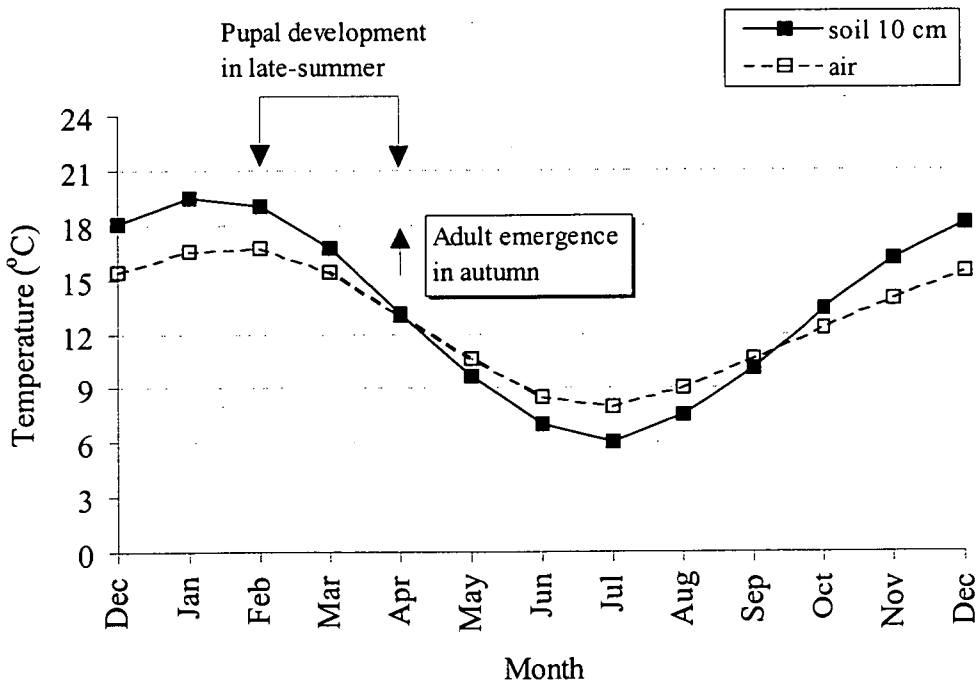


Figure 3.26 Mean monthly soil temperatures (10 cm depth) and air temperatures for Hobart. Soil temperatures are usually above air temperatures in summer and below air temperatures in winter. In most areas of *M. privata*'s distribution, pupal development probably begins some time during summer, thereby enabling adults to emerge in autumn.

continuous development of the pharate adult and thus lead to adult emergence at the earliest opportunity (Fig. 3.25). In contrast, high temperatures (>18°C) seem likely to induce a period of late-pupal aestivation in the pharate adult, thereby delaying the final stages of pupal development and hence, adult emergence.

From the results of this chapter, it is also possible to add further detail to the flow chart of factors influencing the phenology of *M. privata* presented towards the end of Chapter 2 (Fig. 2.13). It is necessary to add steps for stage-1 and stage-2 of adult differentiation after the step representing synchronised onset of pupal development in summer (Fig. 3.27). It is also possible to assign degree-day requirements for egg-larval development and for stage-1 of adult differentiation. Despite finding that pupal duration declines as the season progresses (Chapter 2) and that late-pupal aestivation may occur at temperatures above 18°C (this chapter), the fact that pupae appear to be able to aestivate as well as diapause creates an element of uncertainty about the duration of the pupal stage. It is considered that uncertainty about the duration of the pupal stage is the area most likely to undermine efforts to predict the insect's phenology.

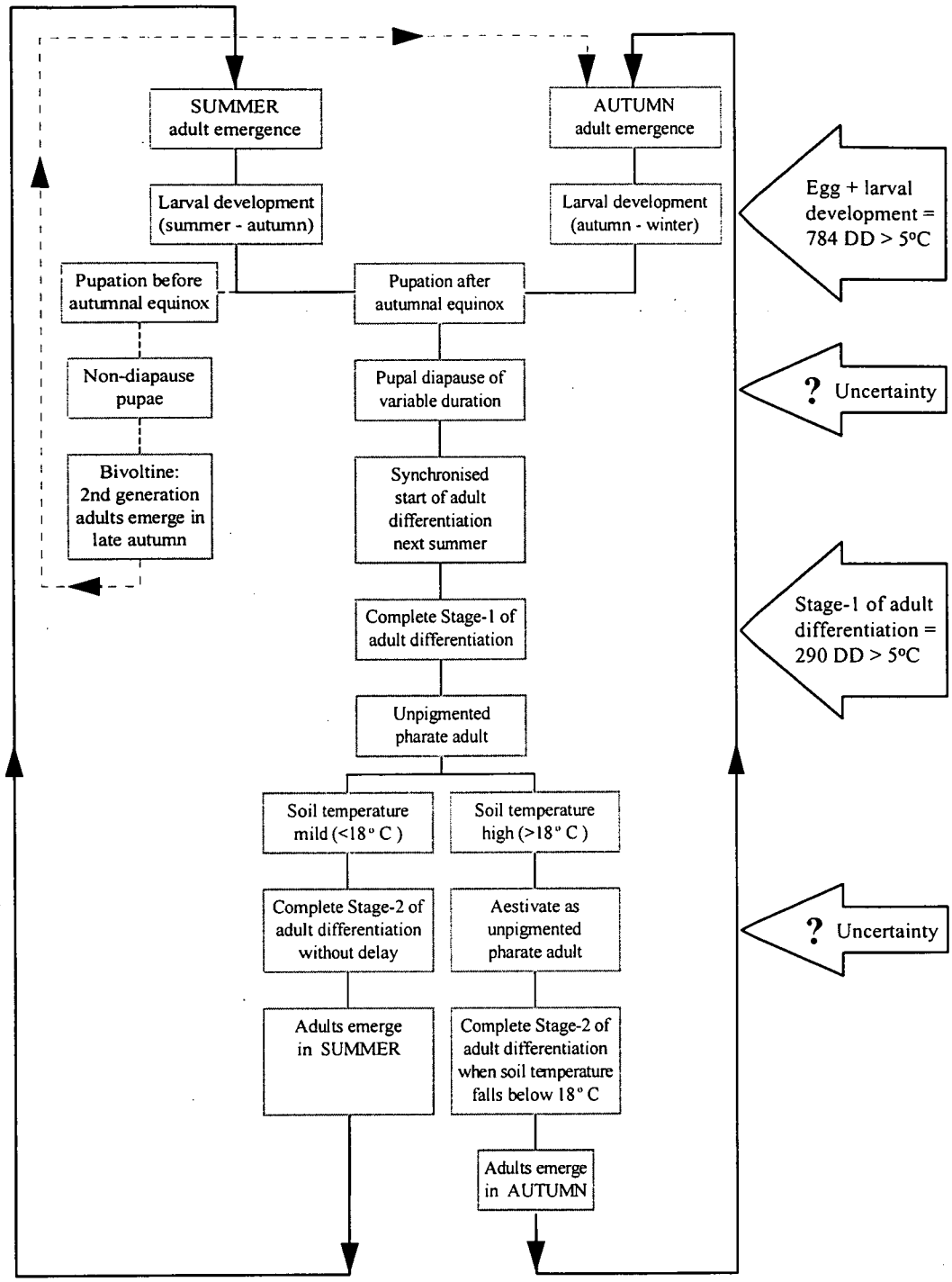


Figure 3.27 Revised flow chart of factors influencing the phenology of *M. privata* in Tasmania. Steps have been added to represent stage-1 and stage-2 of adult differentiation and also to allow for stage-2 of adult differentiation to be delayed by a period of late-pupal aestivation in direct response to soil temperatures above 18°C. Degree-day requirements from oviposition to pupation (= egg + larva) and for stage-1 of adult differentiation are given in the right-hand side of the figure.

4. DEVELOPMENTAL INTERRUPTIONS

4.1 INTRODUCTION

The annual life-cycle of insects usually consists of a period of active growth and development followed by a period of dormancy (Danilevskii, 1961). The majority of insect species are active during the warm-season (i.e. they are 'summer-active') and become dormant during winter (Saunders, 1976; Sauer, *et al.*, 1986). However, some insect species share *M. privata*'s habit of developing during the cool season (i.e. they are 'winter-active') and becoming dormant during summer. Specific terms that refer to the seasonal timing of dormancy are aestivation, autumnal dormancy, hibernation, and vernal dormancy, which refer to dormancy in summer, autumn, winter and spring respectively (Tauber *et al.*, 1984). If dormancy extends over more than one season, the dormancy terms are combined (Pedigo, 1989). For example a dormancy beginning in summer and ending the following winter is termed aestivo-hibernation, with reported examples including some noctuid moths (Oku, 1983) and staphylinid beetles (Topp, 1994). The reverse situation, where dormancy extends from winter to summer, is termed hiberno-aestivation, and so on.

Two distinct types of dormancy are possible in insects. The first is quiescence, where the state of dormancy occurs in direct response to conditions unfavourable for normal development, e.g. low temperature, and ends as soon as conditions become favourable for development again. The second is diapause, where the state of dormancy is induced by token environmental stimuli which, although not detrimental themselves, precede (and hence signal the approach of) conditions unfavourable for development and/or survival (Beck, 1968; Saunders, 1976; Tauber *et al.*, 1984). As noted previously (Section 1.3.2), daylength (or photoperiod) is the most widely-used token stimulus for diapause induction. Other important features of the photoperiodic response are a 'photoperiodic clock' which measures daylength, a

'photoperiodic counter' which counts the number of light-dark cycles, and the 'photosensitive period' or developmental stage(s) sensitive to token stimuli (Saunders, 1976; Denlinger, 1985; Takeda & Skopik, 1997). As noted previously, temperature may modify or over-ride the effect of token stimuli. For example, high temperatures can shorten the sensitive stage to such an extent that it is impossible to accumulate sufficient token stimuli to induce a diapause (Denlinger, 1985). In this situation, diapause would be averted even if insects were reared under daylengths normally inducing diapause.

Although induced by token environmental stimuli, the direct cause of dormancy associated with a diapause is a cessation of neuro-endocrine activity. Hormones necessary for further development are no longer secreted by the neuro-endocrine system, which includes in particular the brain and prothoracic glands (Saunders, 1976; Denlinger, 1985). Development can only resume once the neuro-endocrine system is reactivated after diapause ends and the hormones necessary for development are again secreted (Denlinger, 1985). Since diapause is under hormonal rather than environmental control, it usually occurs irrespective of environmental conditions and development is thus suppressed even under conditions favourable for development.

Beck (1968) summarised the four types of photoperiodic response curves, i.e. curves obtained by plotting the percentage incidence of diapause in a group of experimental insects against daylength (Fig. 4.1). The most important feature of each curve is the 'critical daylength', i.e. the daylength inducing diapause in 50% of the experimental population (Saunders, 1976). The terms used by Beck (1968) to describe the four types of photoperiodic response curve (see caption to Fig. 4.1) are routinely used in the diapause literature to describe the diapause characteristics of a

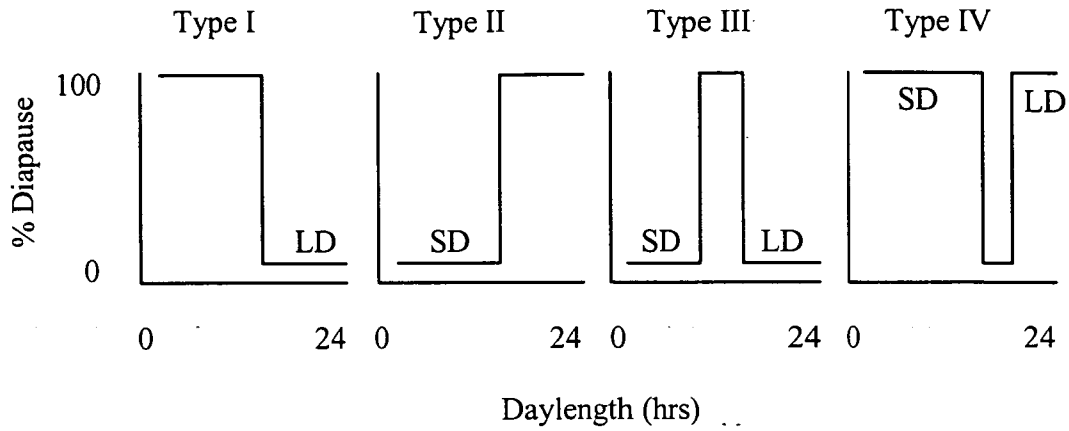


Figure 4.1 Schematic diagram of the four types of photoperiodic response curve summarised by Beck (1968): Type I = long-day response; Type II = short-day response; Type III = short-day–long-day response; Type IV = long-day–short-day response. LD and SD refer to long and short days respectively.

species. For instance, species with a type I response develop continuously in response to long days and hence, are termed 'long-day species'; species with a type II response develop continuously in response to short days and hence are termed 'short-day species' and so on. Furthermore, some insects respond to changing daylength rather than absolute daylength and respond differently to a decreasing daylength in autumn as against an increasing daylength during spring. For instance, Australian plague locust (*Chortoicetes terminifera*) females that experience a decrease in daylength during development in autumn lay a high percentage of diapausing eggs, whereas females that experience an increase in daylength during development in spring and summer lay non-diapause eggs (Wardhaugh, 1986).

The terms used for type I-III photoperiodic response curves are based on the daylengths at which a positive developmental response occurs (i.e. continuous or non-diapause development) (see Fig. 4.1). In contrast, the term used for the type IV curve (long-day–short-day response) appears to be based on the daylengths at which a negative developmental response (i.e. diapause induction) occurs (Fig. 4.1). Thus, the terminology is inconsistent and, in this author's opinion, enables curve types III

and IV in particular to be easily confused. To be consistent with the terminology used for response types I-III, the term for Beck's type-IV response should refer to the positive developmental response (non-diapause) that occurs only over a narrow range of long daylengths in that response type. Danilevskii (1961) and Saunders (1976) refer to the type IV response as an 'intermediate' response type. However, a more appropriate, and perhaps less ambiguous term for the type IV response could be a 'restricted long-day response'.

Diapause is a major factor regulating the annual life-cycle of many temperate insects (Tauber *et al.*, 1984; Bradshaw, 1986). Summer-active insects that enter winter diapause usually develop continuously under long days and enter diapause in response to short days, i.e. they are long-day species (Saunders, 1976). Alternatively, winter-active insects that enter summer diapause usually develop continuously under short days and enter diapause in response to long days, i.e. they are short-day species (Saunders, 1976). Therefore, since *M. privata* is predominantly a winter-active species throughout its distribution, we might originally expect it to develop continuously under short days and diapause in response to long days. However, the results of Chapter 2 suggested that the opposite is true. *M. privata* entered a pupal diapause if larvae developed in autumn and winter when days were short, and averted a pupal diapause if larvae developed in summer when days were long (see Fig. 2.11). Such behaviour typifies a summer-active rather than a winter-active species. Furthermore, the fact that *M. privata* routinely develops during winter on mainland Australia and in lowland areas of Tasmania indicates that a hibernal diapause is unnecessary for winter survival in those areas. This raises an interesting question in relation to *M. privata* phenology: why should the species enter a hibernal diapause (induced by short days) when it is not needed for winter survival?

4.2 AIMS AND OBJECTIVES

The aim of the previous chapter was to relate development times of the immature stages of *M. privata* to temperature while, at the same time, producing laboratory cohorts of pupae under different temperatures and daylengths that could subsequently be used to further study pupal diapause. Pupae with a range of thermal and photoperiodic histories were finally produced in Experiment 3 of Chapter 3, in which pupae were produced under five different environments (see Table 4.1). The objective of this chapter is to examine the pattern of adult emergence from those pupae in order to verify or disprove the hypothesis that pupal diapause in *M. privata* is induced by short daylengths experienced during larval development. As noted in Chapter 3, short pupal periods following larval development under long daylengths and extended pupal periods following larval development under short daylengths would provide evidence supporting the diapause hypothesis.

4.3 METHODS

4.3.1 Source of pupae and pupal rearing conditions

Pupae produced under the five larval rearing environments given in Table 4.1 were incubated at a standard temperature of 15°C, chosen because it falls between the lower developmental threshold of about 5°C and a late-pupal aestivation threshold of approximately 18°C found for *M. privata* pupae in Experiment 4 of Chapter 3. Pupae were held in inverted vial-lids inside transparent plastic boxes as before (Fig. 2.3). Since eggs had originally been allocated to different temperatures at the same time, pupation occurred sequentially from the highest to the lowest temperature (Table 4.1). Pupae forming at 24.0°C and 21.0°C (= 24/18°C, 12L:12D) (i.e. above 18°C) remained at those temperatures for mean durations of 35 and 29 days respectively, while larvae reared at 15.0°C and 16.8°C completed their development (Table 4.1). Thereafter, pupae from those four temperatures were weighed, sexed

Table 4.1 The origin of pupae used to study diapause in this chapter. Pupae from five larval rearing environments were incubated at a standard temperature of 15°C. Approximately half of the experimental pupae from each larval rearing environment were incubated under a 16 hr daylength (16L:8D), while the other half were incubated in darkness (0L:24D) (see Fig. 4.2). This gave a total of ten 'treatments'. Treatment numbers are given in parentheses in the last two columns of the table. Treatments 1-5 refer to 'Illuminated pupae', while treatments 6-10 refer to 'Dark pupae'.

Larval Rearing Environment:		Mean Date of Pupation (1996)	Mean number of days from pupation to placement at 15°C (days)	Number of Pupae:			Number of Pupae Placed at:	
Temperature (°C)	Photoperiod (L : D)			Initial	Excluded	Remain for use	15°C 16L:8D = ILLUMINATED PUPAE	15°C 0L:24D = DARK PUPAE
24.0	0 : 24	3 Aug.	35	28	2	26	13 (1)	13 (6)
24/18 [a]	12 : 12	10 Aug.	29	42	2	40 [d]	15 (2)	15 (7)
16.8 [b]	8 : 16	1 Sep.	8	51	6	45	23 (3)	22 (8)
15.0	16 : 8	6 Sep.	3	60	15	45	20 (4)	25 (9)
11.5	16 : 8	27 Oct.	3	30	0	30 [d]	9 (5)	11 (10)
Total				211	25 [c]	186	80	86

[a] The mean daily temperature of the fluctuating regime was 21°C.

[b] All larvae developing at 16.8°C (8L:16D) were transferred to 15°C (16L:8D) late in the fourth instar due to equipment shortages. Despite completing larval development and pupating at 15°C (16L:8D), the resulting group of pupae is referred to throughout the chapter as though pupation occurred at 16.8°C, thus distinguishing the group from that which developed entirely at 15°C (16L:8D).

[c] 25 pupae were excluded from the experimental group because they initiated adult differentiation before the start of the experiment.

[d] Ten pupae were left as controls at 21°C and also at 11.5°C to see if development would begin within 45 days at those temperatures.

and examined for visible signs of development under a stereoscope. Pupae formed last at 11.5°C and were weighed, sexed, examined for development and transferred to 15.0°C within three days of pupation (Table 4.1). Twenty-five pupae initiated adult differentiation before the start of the experiment and were therefore excluded from the experimental group (Table 4.1). This procedure ensured that no pupae in the experimental group had begun developing prior to placement at 15°C.

Half the pupae from each of the five larval rearing environments were incubated under a 16 hr daylength, while the other half were incubated in darkness (Fig. 4.2), giving a total of ten treatments (Table 4.1). This was done to test whether *M. privata* pupae may be sensitive to photoperiodic influences during the pupal stage, a phenomenon reported previously in some other lepidopteran species, e.g. the noctuids *Mamestra brassicae* (reviewed in Beck, 1968) and *Helicoverpa punctigera* (Cullen & Browning, 1978) which are both sensitive to temperature and photoperiodic influences during the early part of the pupal stage.

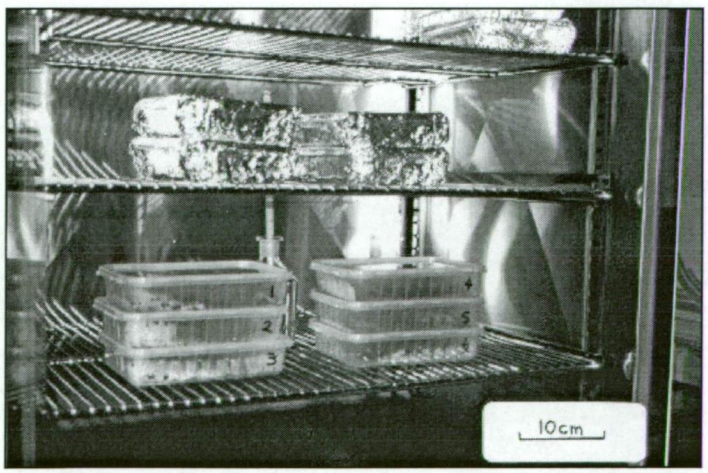


Figure 4.2 During incubation, pupae were held in transparent plastic boxes, which exposed them to a 16 hr daylength. When darkness was required the boxes were wrapped in aluminium foil.

4.3.2 Recognition of diapause

As noted in the introduction to this chapter, quiescence occurs in direct response to unfavourable conditions whereas diapause may occur even under favourable conditions because it is under hormonal control. Since the standard temperature of 15°C used to incubate pupae in this chapter fell within a temperature range that is favourable for continuous pupal development (5-18°C), non-development at 15°C is interpreted in this study as an indication of pupal diapause. It was also determined previously that at 15°C, pupae took a mean duration of 30.9 ± 1.8 days to develop to an unpigmented pharate adult (Table 3.15) and 52.2 ± 1.6 days to develop completely (Table 3.12). Based on these developmental times, pupae were incubated at 15°C for an intermediate period of 45 days, after which they were examined individually under a stereoscope. Pupae were classed as 'non-diapause' if development had begun and could be classified according to Fig. 3.16, or 'diapause' if pupae still possessed eye-spots and thus resembled Fig. 2.4a. Non-diapause and diapause pupae were then reared to adult eclosion at 15°C and total pupal durations (at 15°C) recorded. Finally, the incidence of diapause and the duration of the pupal stage were assessed in relation to larval rearing environment and pupal lighting conditions.

It must be noted that although pupal duration and the incidence of diapause were investigated among pupae produced under five larval rearing environments, only the short-day regime (8L:16D) at 16.8°C and the long-day regime (16L:8D) at 15.0°C were specifically designed to test the diapause hypothesis. The slight temperature difference between the short day and long day regimes was unfortunate and unplanned. The short day incubator maintained a slightly higher temperature despite being programmed with the same temperature setting as the long day incubator. To simplify the interpretation of results, the temperature difference of

1.8°C during larval development was assumed to have had no effect on the incidence of diapause or subsequent pupal duration in the two groups.

4.3.3 Statistical analysis

A range of statistical tests were used in the analysis of the pupal data:

- Firstly, before examining pupal duration or the incidence of diapause, mean pupal weights were examined in relation to pupal sex and larval rearing temperature. T-tests were used to compare mean weights of male and female pupae produced at each temperature. Then, one-way ANOVA tests were used to determine whether mean pupal weights varied with temperature within each sex.
- Following the examination of pupal weights, Chi-Square tests were used to test the null hypothesis that the proportions of diapause and non-diapause pupae were equal in each of the ten treatments given in Table 4.1. Under the null hypothesis, 50% of pupae from each treatment were expected to be in each developmental category. Contingency tables were used next to test whether the proportion of pupae in each developmental category (diapause or non-diapause) varied with respect to larval rearing environment and pupal lighting conditions. First, a 10 x 2 contingency table tested whether the proportion in each category varied over all ten treatments. Second, separate 5 x 2 contingency tables for treatments 1-5 and 6-10 tested whether the proportion in each category varied among the lit and dark treatments respectively. Third, separate 2 x 2 contingency tables for each of the five larval rearing temperatures tested whether the proportion in each category varied depending on whether or not pupae were illuminated during incubation.
- Finally, frequency distributions were used to examine the range in pupal duration, and thereby the pattern of adult emergence, in each treatment.

4.4 RESULTS

4.4.1 Pupal weight in relation to sex and larval rearing temperature

The mean weights of *M. privata* pupae in relation to sex and larval rearing temperature are presented in Table 4.2. Females were significantly heavier than males at each temperature in the Tarraleah family and also in the Cobram family, which tended to have consistently lower mean weights for both sexes (Table 4.2). The mean weight of Tarraleah females ranged from 226.5 to 260.0 mg, compared with 176.3 to 206.5 mg for Tarraleah males, while the mean weight of Cobram females ranged from 182.9 to 222.7 mg compared with 129.6 to 178.7 mg for Cobram males (Table 4.2).

The mean weight of females varied depending on larval rearing temperature in the Tarraleah family ($F_{4,108} = 4.55$, $p < 0.01$), but not in the Cobram family ($F_{3,28} = 1.56$, $p = 0.22$). Tarraleah females produced at 24.0°C were significantly heavier than sibling female pupae produced at 11.5-21.0°C, over which temperature range mean weights of Tarraleah females were not significantly different (Table 4.3a). Meanwhile, the mean weight of males varied depending on larval rearing temperature in both the Tarraleah family ($F_{4,103} = 7.09$, $p < 0.01$) and the Cobram family ($F_{3,26} = 6.66$, $p < 0.01$). Tarraleah males produced at 24.0°C were significantly heavier than sibling male pupae produced at lower temperatures, while males produced at 16.8°C were significantly lighter than those produced at 11.5 and 15.0°C (Table 4.3a). Cobram males produced at 15.0°C were significantly lighter than sibling male pupae produced at 11.5°C and 21.0°C (Table 4.3b).

The main findings were that females were usually heavier than males and that the highest rearing temperature used was not detrimental to larval growth, since the heaviest pupae were produced at 24°C in the Tarraleah family, albeit in darkness.

Table 4.2 The mean weight of *M. privata* pupae in relation to larval rearing temperature and pupal sex. Separate results are shown for the two families used in the developmental study. Significance levels are based on T-tests comparing means (i.e. males vs. females) in each row. * = $p < 0.05$, ** = $p < 0.01$.

Origin of Family	Temperature for Egg-Larval Development (°C)	Pupal Weight (mg)								
		Males				Females				Significance
		Mean	SE	n	Range	Mean	SE	n	Range	Level
Tarraleah (Tasmania)	24.0	206.5	3.1	12	191 - 230	260.0	6.4	18	200 - 299	**
	21.0	186.1	4.9	23	143 - 230	226.5	7.3	21	159 - 269	**
	16.8	176.3	3.0	26	149 - 207	231.9	3.8	26	189 - 262	**
	15.0	184.9	2.6	30	156 - 222	238.9	4.6	35	161 - 277	**
	11.5	192.2	3.9	17	159 - 218	233.2	6.3	13	191 - 267	**
	Overall	186.7	1.8	108	143 - 230	237.7	2.6	113	159 - 299	**
Cobram (Victoria)	21.0	174.0	4.4	9	144 - 185	202.4	10.2	8	163 - 243	*
	16.8	159.1	12.8	5	127 - 185	212.4	13.2	10	153 - 273	*
	15.0	129.6	11.7	9	83 - 176	182.9	16.6	6	120 - 224	*
	11.5	178.7	6.5	7	158 - 203	222.7	10.25	8	169 - 253	**
	Overall	159.3	5.7	30	83 - 176	206.9	6.5	32	120 - 273	**

Table 4.3 Summary table showing whether or not the mean weight of *M. privata* pupae from a) the Tarraleah family and b) the Cobram family differed significantly between temperatures. The top-right half of each matrix refers to comparisons between females, the bottom-left half (shaded) to comparisons between males. Probabilities are based on T-tests. * = $p < 0.05$, ** $p < 0.01$, all others not significant.

a) Tarraleah

Females

Temperature (°C)	Wt (mg)	11.5	15.0	16.8	21.0	24.0	Females
11.5	192.2		0.51	0.85	0.53	**	233.2
15.0	184.9	0.12		0.27	0.14	**	238.9
16.8	176.3	**	*		0.49	**	231.9
21.0	186.1	0.37	0.82	0.09		**	226.5
24.0	206.5	*	**	**	**		260.0
	Males	192.2	184.9	176.3	186.1	206.5	

Males

b) Cobram

Females

Temperature (°C)	Wt (mg)	11.5	15.0	16.8	21.0	Females
11.5	178.7		0.052	0.56	0.18	222.7
15.0	129.6	**		0.19	0.31	182.9
16.8	159.1	0.17	0.14		0.57	212.4
21.0	174.0	0.55	**	0.2		202.4
	Males	178.7	129.6	159.1	174.0	

Males

4.4.2 Incidence of pupal diapause after 45 days at 15°C

Unless stated, the following results refer only to pupae from the Tarraleah family. The results from the Cobram family were not analysed in any great detail because that family produced no pupae at 24°C and fewer than twenty pupae at each of the remaining four temperatures. This meant that after dividing Cobram pupae into illuminated and dark treatments, expected cell counts for diapause and non-diapause pupae in Chi-Square tests were always less than 5, considered the minimum number required for a valid test (Mendenhall, 1983, p. 517). Despite this, results from the Cobram family are included where appropriate for comparative purposes.

The number and percentage of diapause and non-diapause pupae present in each of the ten treatments after 45 days of incubation at 15°C are presented in Table 4.4. The proportion of pupae in each developmental category departed significantly from 50% in treatments 1-4 and treatments 7,8 and 10, but not in treatments 5,6 and 9 (Table 4.4). The proportion in each category varied significantly between the ten treatments ($\chi^2_9 = 92.20$, $p < 0.01$), and also among treatments 1-5 (illuminated) ($\chi^2_4 = 49.05$, $p < 0.01$) and treatments 6-10 (incubated in darkness) ($\chi^2_4 = 31.07$, $p < 0.01$). The incidence of diapause ranged from 0-100% among all ten treatments, from 0-100% among treatments 1-5 and from 44-100% among treatments 6-10 (Table 4.4). No 'control' pupa remaining at 11.5°C developed and only one control pupa remaining at 21.0°C developed (Table 4.4). The behaviour of control pupae at 11.5° and 21.0°C was therefore very similar to that exhibited by pupae produced at those temperatures and then incubated in darkness at 15°C.

Comparisons between 'illuminated' and 'dark' pupae produced at each larval rearing temperature determined that the ratio of diapause to non-diapause pupae varied significantly with pupal lighting conditions in four out of five cases. The incidence of diapause among pupae produced at 11.5, 15.0, 21.0 and 24.0°C was 22% or lower among illuminated pupae compared with 44% or higher among pupae incubated in darkness (Fig. 4.3). In contrast to the previous result, pupal lighting conditions had no significant effect on the incidence of diapause among pupae produced under an 8 hr daylength at 16.8°C. Those pupae exhibited a very high incidence of diapause (95-100%) regardless of pupal lighting conditions (Fig. 4.3).

Table 4.4 The number and percentage of non-diapause and diapause pupae after 45 days of incubation at 15°C following egg-larval development under five different environments. Significance levels are based on Chi-Square tests comparing the number of diapause and non-diapause pupae in each treatment. * = $p < 0.05$; ** = $p < 0.01$; ns = not significant. The two extra rows below the table refer to control pupae remaining at 11.5 and 21°C.

Treatment	Egg-Larval development		Pupal Development	Number of pupae:					Significance	Percentage of pupae:	
	Temperature (°C)	Photoperiod (L : D)		Initial	Dead	Final	Non-diapause	Diapause		Non-diapause %	Diapause %
1	24.0	0 : 24	15°C 16L:8D	13	1	12	12	0	**	100	0
2	24/18	12 : 12	"	15	1	14	11	3	*	79	21
3	16.8	8 : 16	"	23	1	22	0	22	**	0	100
4	15.0	16 : 8	"	20	1	19	16	3	**	84	16
5	11.5	16 : 8	"	9	0	9	7	2	ns	78	22
Total Lit				80	4	76	46	30		61	39
6	24.0	0 : 24	15°C Dark	13	0	13	7	6	ns	54	46
7	24/18	12 : 12	"	15	1	14	0	14	**	0	100
8	16.8	8 : 16	"	22	0	22	1	21	**	5	95
9	15.0	16 : 8	"	25	0	25	14	11	ns	56	44
10	11.5	16 : 8	"	11	0	11	0	11	**	0	100
Total Dark				86	1	85	22	63		26	74
Control - 21.0	24/18	12 : 12	24/18°C 12L:12D	10	0	10	1	9	*	10	90
Control - 11.5	11.5	16 : 8	11.5°C 16L:8D	10	0	10	0	10	**	0	100

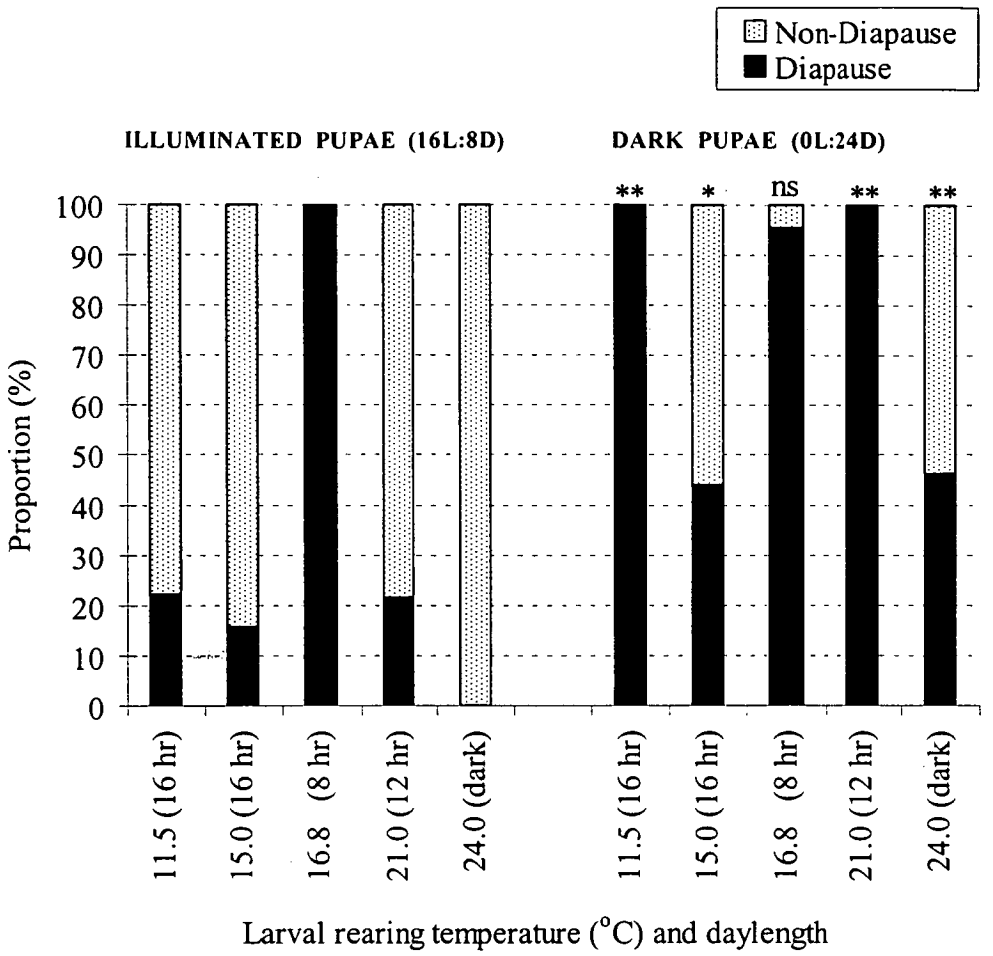


Figure 4.3 The incidence of diapause in *M. privata* pupae after 45 days of incubation at 15°C in relation to larval and pupal rearing environments. Significance levels are based on 2 x 2 contingency tables comparing the proportion of diapause and non-diapause pupae in illuminated and dark treatments among pupae produced at the same temperature (e.g. 11.5°C illuminated vs. 11.5°C dark). * = $p < 0.05$, ** = $p < 0.01$, ns = not significant.

4.4.3 Pupal duration in relation to larval and pupal rearing environments

The mean pupal durations among non-diapause and diapause pupae of *M. privata* at 15°C in each of the ten treatments are presented in Table 4.5. The patterns of adult emergence over time in the ten treatments are presented in Fig. 4.4. The mean (±SE) pupal duration among non-diapause pupae was 60.9 ± 1.5 days (n = 66, range = 44-105 d) while the mean pupal duration among diapause pupae was over three times longer at 215.6 ± 7.1 days (n = 72, range = 119-366 d) (Table 4.5). Therefore, the conspicuous peaks of adult emergence at about 60 days in treatments 1, 2, 4, 6 and 9

Table 4.5 The mean duration of the pupal stage of *M. privata* at 15°C among diapause and non-diapause pupae. Pupae were produced under five different environments, then incubated under a 16 hr daylength or in darkness. Means in the two rows labelled 'Illuminated pupae' and 'Dark pupae' that are followed by different letters are significantly different ($p < 0.01$) based on T-tests.

Treatment	Egg-Larval development		Pupal Development	Pupal Duration (days)							
				Non-Diapause Pupae				Diapause Pupae			
	Temperature (°C)	Photoperiod (L:D)		Mean	SE	n	Range	Mean	SE	n	Range
1	24.0	0 : 24	15°C 16L:8D	59.8	1.6	12	54 - 71	-	-	-	-
2	24/18	12 : 12	"	59.0	1.5	10	54 - 68	306.0	0.0	1	306
3	16.8	8 : 16	"	-	-	-	-	172.2	2.9	17	157 - 195
4	15.0	16 : 8	"	52.3	2.1	16	44 - 79	205.0	0.0	1	205
5	11.5	16 : 8	"	87.3	5.5	7	62 - 105	145.0	26.0	2	119 - 171
Illuminated pupae				61.2 [a]	2.1	45	44 - 105	177.6 [b]	7.5	21	119 - 306
6	24.0	0 : 24	15°C Dark	60.7	3.4	6	45 - 68	194.0	29.9	5	137 - 304
7	24/18	12 : 12	"	-	-	-	-	271.0	12.3	12	186 - 316
8	16.8	8 : 16	"	54.0	0.0	1	-	190.4	9.6	20	160 - 366
9	15.0	16 : 8	"	60.3	1.1	14	54 - 68	316.9	13.3	7	283 - 364
10	11.5	16 : 8	"	-	-	-	-	220.6	6.1	7	199 - 242
Dark pupae				60.1 [a]	1.2	21	45 - 68	231.2 [c]	8.7	51	137 - 366
Overall				60.9	1.5	66	44 - 105	215.6	7.1	72	119 - 366

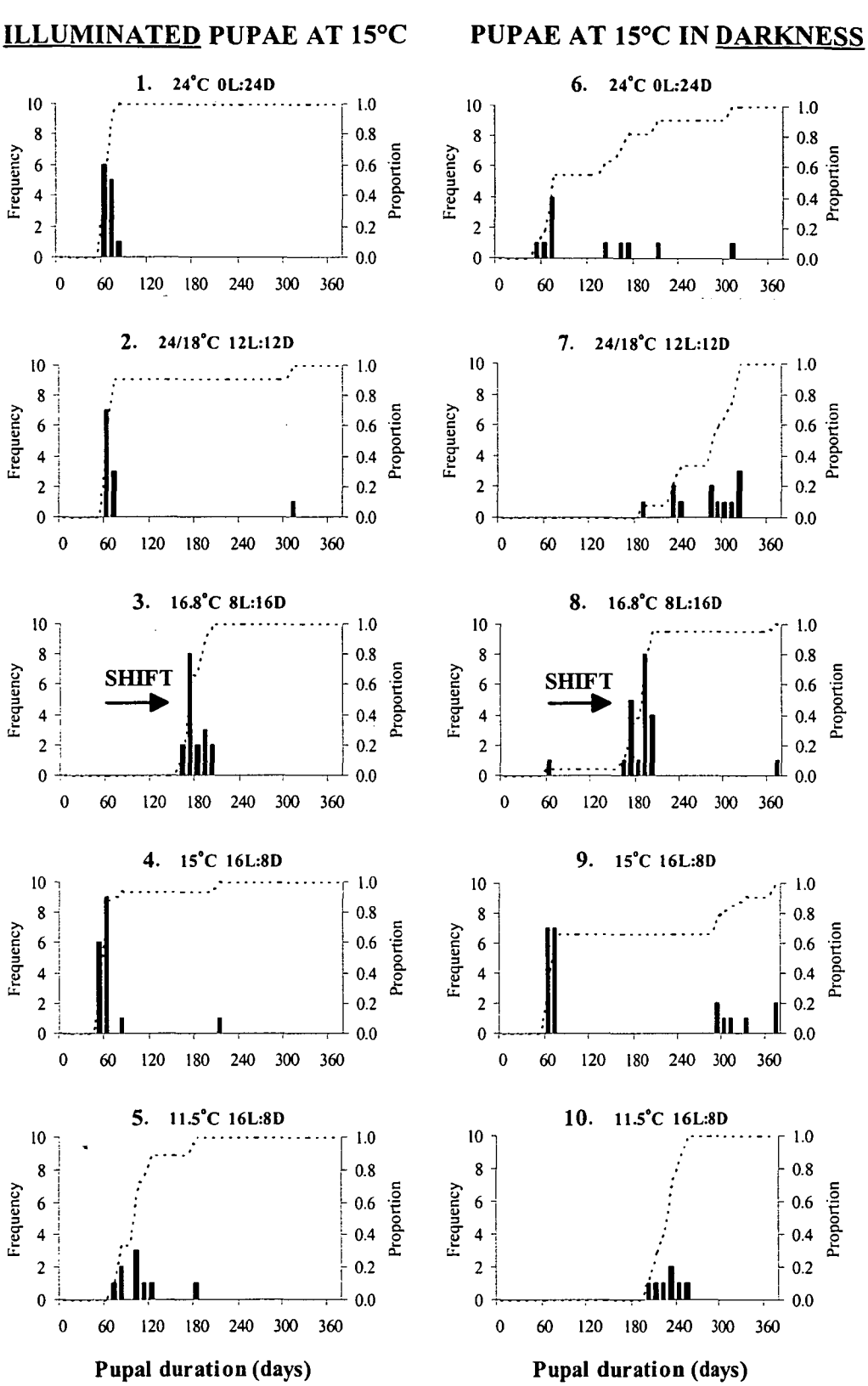


Figure 4.4 Emergence patterns of adult *M. privata* at 15°C in relation to larval rearing environment and pupal lighting conditions. Treatment numbers and thermal and photoperiodic histories of pupae are given above each histogram. The left-hand column refers to pupae incubated under a 16 hr daylength, the right-hand column to pupae incubated in darkness. The horizontal arrows in treatments 3 and 8 refer to a shift from non-diapause to diapause pupae in response to short daylengths experienced during larval development.

evident in Fig. 4.4 must be attributed to adult emergence from non-diapause pupae, whereas peaks of adult emergence at around 180 d (treatments 3 and 8), 240 d (treatment 10) and 300 d (treatments 7 and 9) must be attributed to adult emergence from diapause pupae. In the remaining treatment (treatment 5), seven adults emerged from non-diapause pupae after 62-105 d and two adults emerged from diapause pupae after 119 and 171 days (Fig. 4.4).

The key finding from the results reported in this section was that a major shift from a non-diapause pathway to a diapause pathway was associated with different daylengths experienced during larval development. The majority of pupae produced under long days (16L:8D) at 15.0°C averted a diapause (treatments 4 and 9 in Fig. 4.4) while the majority of pupae produced under short days (8L:16D) at 16.8°C entered a pupal diapause (treatments 3 and 8 in Fig. 4.4).

Similar patterns of adult emergence were exhibited by the Cobram family. An early peak of adult emergence from non-diapause pupae occurred after about 60 days, while most diapause pupae from the Cobram family emerged after about 180 days (Fig. 4.5). The main difference was observed among Cobram pupae produced at 21°C and then incubated in darkness. Three pupae in that group averted pupal diapause (c.f. no non-diapause pupae in the equivalent Tarraleah group) while adults emerged earlier from the four diapause pupae (170-210 days in the Cobram family c.f. 180-320 days in the Tarraleah family) (Fig. 4.5). Similar adult emergence patterns in treatments 2, 3 and 8 in particular suggested that there were no major differences in diapause characteristics between the two families.

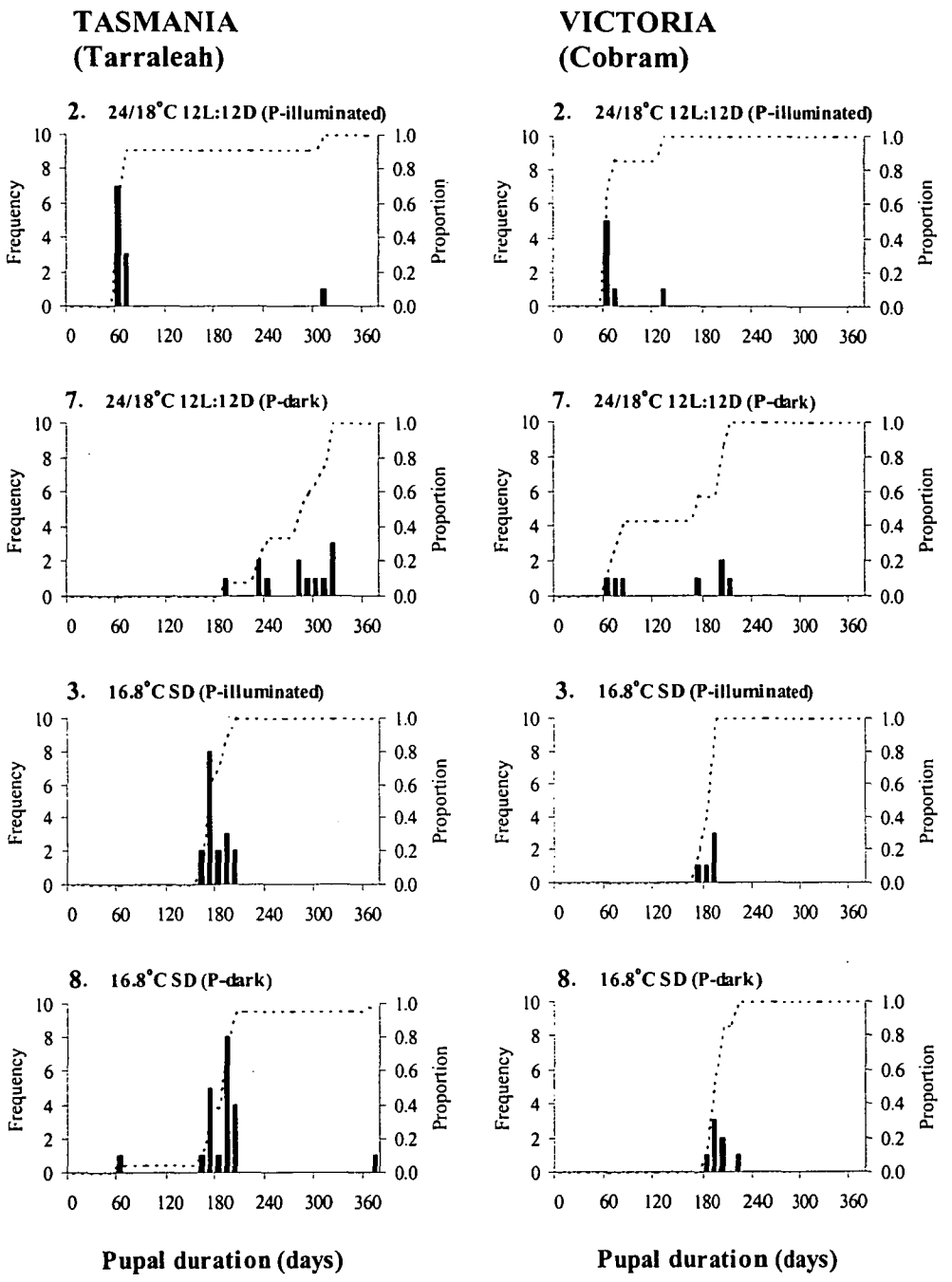


Figure 4.5 A comparison of adult emergence patterns from *M. privata* pupae originating from different localities in southern Australia. The left-hand column refers to pupae originating from Tarraleah in central Tasmania, the right-hand column to pupae originating from Cobram in northern Victoria. Treatment numbers and thermal and photoperiodic histories of pupae are given above each histogram. All pupae were incubated at 15°C. 'P-illuminated' refers to pupae incubated under a 16 hr photoperiod. 'P-dark' refers to pupae incubated in darkness.

4.4.4 Unexpected results

Two events took place during the larval rearing process that, despite initial concerns, ultimately proved to be fortunate for they yielded unexpected results. Firstly, the need to transfer larvae from short days at 16.8°C to long days at 15°C late in the 4th instar (see footnote *b* in Table 4.1) yielded preliminary information about the diapause induction process during larval development. Larvae that developed to late-L4 under short days produced predominantly diapause pupae despite the fact that they completed their development under long day conditions (shown schematically in Fig. 4.6). This result suggested that diapause was induced before larvae reached the 5th instar and that subsequent exposure to long days during the 5th instar and the pupal stage did not reverse the induction of diapause.

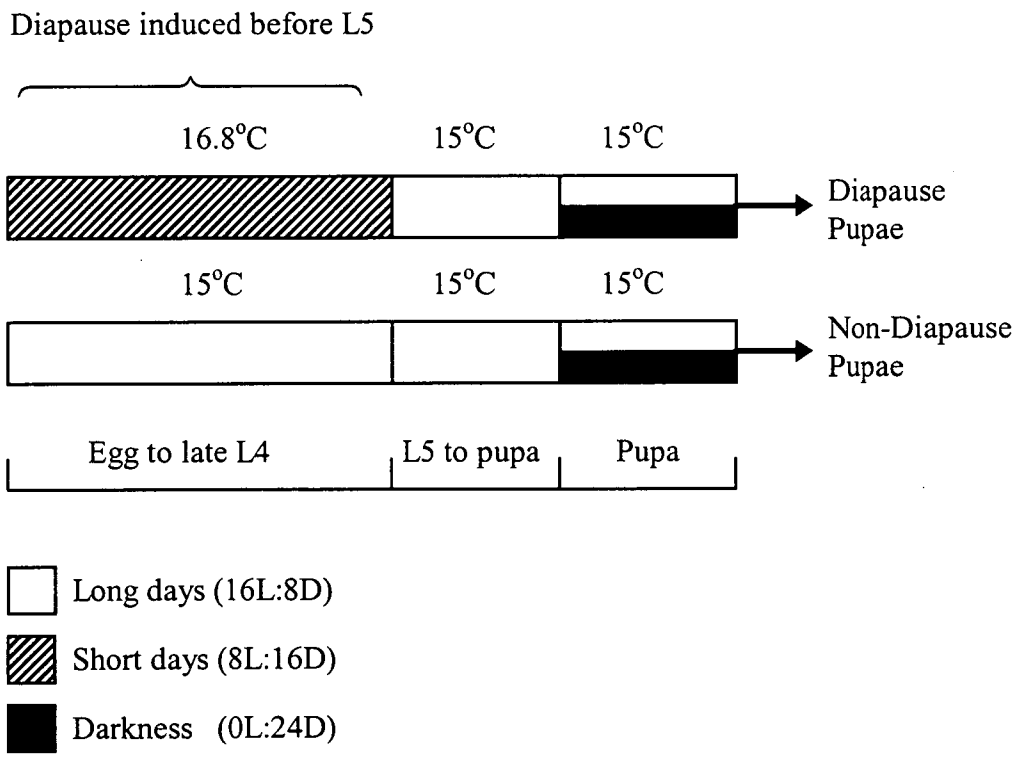


Figure 4.6 Schematic diagram showing when pupal diapause was induced during the larval stage of *M. privata*. Diapause was induced by short days before the 5th instar in laboratory-reared larvae. Subsequent exposure to long days during the 5th instar did not reverse the induction of diapause.

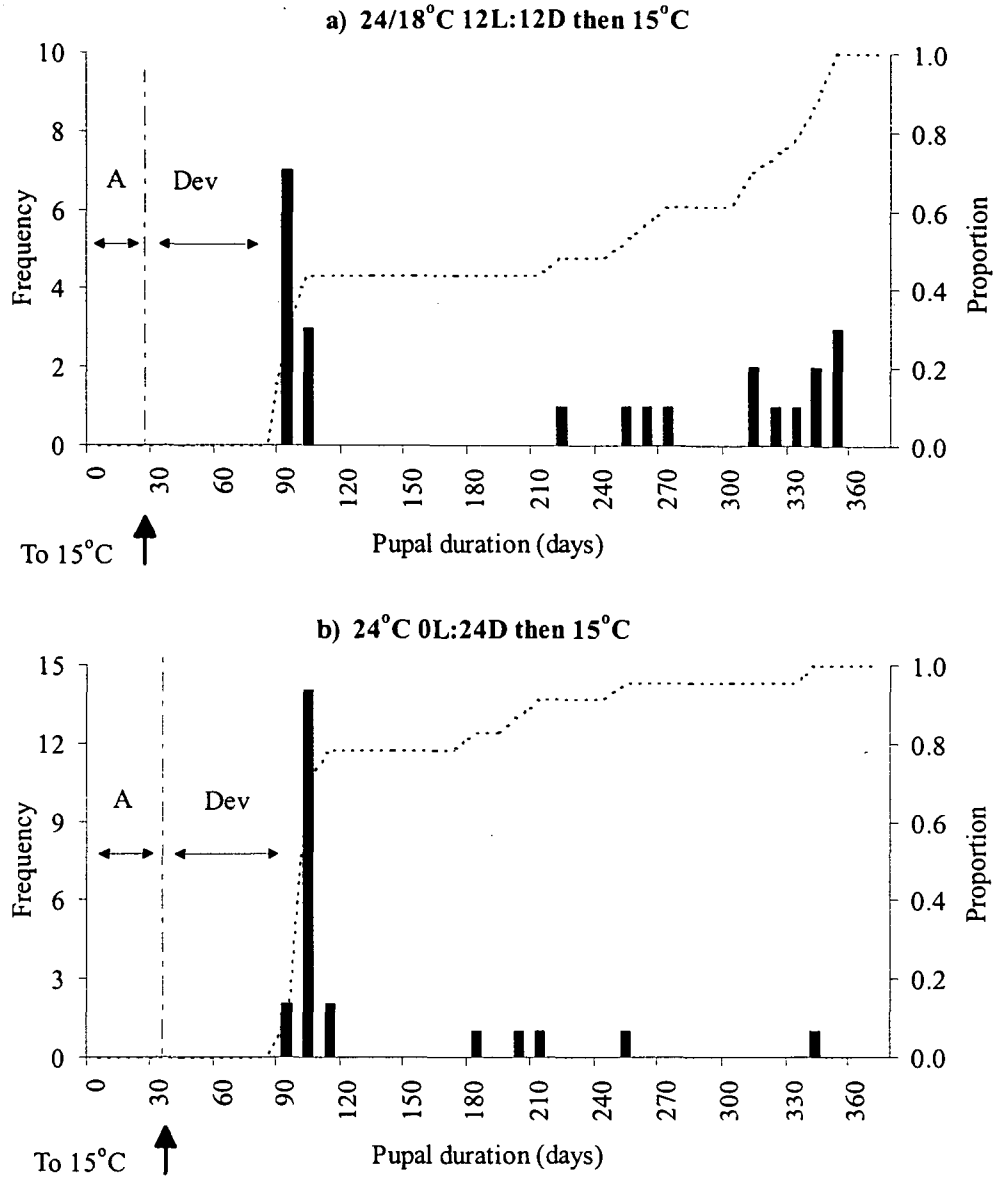


Figure 4.7 The pupal duration of *M. privata* after larvae were reared at 24/18°C 12L:12D and 24°C 0L:24D. Newly formed pupae spent an average of 29 days at 24/18°C (a) and 35 days at 24°C (b) before being placed at 15°C. Note the usual 60-70 day developmental period (= 'Dev') after pupae were placed at 15°C. The letter 'A' refers to periods of aestivation lasting 29 and 35 days at 21° and 24°C respectively before pupae were placed at 15°C. Pupal durations exceeding 120 days indicate diapause pupae.

The second unexpected result was found as a consequence of pupae forming earlier at higher temperatures than at lower temperatures after eggs were originally placed at different temperatures at the same time. Pupae forming at 21° and 24°C remained at those temperatures for mean durations of about 29 and 35 days

respectively before being placed at 15°C (Table 4.1). The initial examination (before placing pupae at 15°C) revealed that despite remaining at 21° and 24°C for the above periods, only two pupae were developing at each of those temperatures (Table 4.1). However, once pupae produced at 21° and 24°C were placed at 15°C, a peak of adult emergence was observed in both groups about 60-70 days later, or 90-100 days after pupation (Fig. 4.7). Given that approximately 60-70 days were required for non-diapause pupal development at 15°C, it is considered that adults that emerged after 90-100 days were from non-diapause pupae that had aestivated for about 30 days at 21° and 24°C before developing to adult eclosion in the usual period of 60-70 days after placement at 15°C (Fig. 4.7). The implications of these unexpected findings for *M. privata* phenology are considered in the discussion.

4.5 DISCUSSION

4.5.1 Induction of pupal diapause in M. privata

The primary objective of this chapter was to verify or disprove the hypothesis that pupal diapause in *M. privata* is induced by short daylengths experienced during larval development and averted by long daylengths. Hence, it was necessary to determine whether the incidence of pupal diapause, defined in this study as non-development of pupae after 45 days of incubation at 15°C, was high among pupae produced under short daylengths and low among pupae produced under long daylengths. As noted previously, short pupal periods following larval development under long daylengths and extended pupal periods following larval development under short daylengths would also provide evidence supporting the diapause hypothesis.

Experimental results revealed that a very high incidence of pupal diapause (95-100%) (Fig. 4.3) and long pupal durations (160-200 d) (Fig. 4.4) were usually

associated with larval development under short days at 16.8°C, whereas a relatively low incidence of pupal diapause (16-44%) (Fig. 4.3) and short pupal durations (60-70 d) (Fig. 4.4) were usually associated with larval development under long days at 15.0°C. Thus, assuming that the temperature difference of 1.8°C between the short-day and long-day environments had no effect on the incidence of diapause in the two groups, the above results provide strong evidence supporting the hypothesis that pupal diapause in *M. privata* is induced by short daylengths experienced during larval development.

Underlining the diapause inducing effect of short daylengths was the fact that pupae produced under short days at 16.8°C were the only group to exhibit a high incidence of diapause (95-100%) regardless of pupal lighting conditions. Pupae produced at 11.5, 15.0, 21.0 and 24.0°C exhibited a significantly higher incidence of diapause in darkness (44-100%) than when illuminated (0-22%) (Fig. 4.3). The latter result suggests that *M. privata* pupae were sensitive to photoperiodic influences and that illumination stimulated pupal development whereas darkness had an inhibitory effect. This result contradicted an earlier finding in Chapter 2 that pupal illumination did not cause early adult emergence in the laboratory (Table 2.6). It now appears that pupae can respond to photoperiodic influences under some circumstances. For example, pupae produced under long-days at 11.5°C tended to avert a diapause when illuminated, but enter a diapause when incubated in darkness (Fig. 4.3). An almost identical pattern was observed among pupae produced at 21.0°C (Fig. 4.3). Such results might reflect mixed messages received during larval development (e.g. long daylengths concurrent with low temperatures). Perhaps pupae were not committed to either developmental pathway because of such mixed messages and randomly took a diapause or non-diapause pathway after pupation.

Previous studies have reported a similar phenomenon in pupae of other lepidopteran species. Beck (1968) noted that aestival diapause in pupae of the cabbage moth *Mamestra brassicae* was "determined by the photoperiod and temperature conditions experienced during the first few days after pupation", with long daylengths and high temperatures inducing aestival diapause. However, other studies have shown that aestival diapause in *M. brassicae* is induced by long days and high temperatures experienced during larval development (Sauer *et al.*, 1986; Grüner & Sauer, 1988; Grüner & Masaki, 1994). Moreover, Sauer et al. (1986) argue that aestival diapause in *M. brassicae* is not a true form of diapause but a modified form of non-diapause development. Meanwhile, Cullen and Browning (1978) found that *Helicoverpa punctigera* pupae were sensitive to temperature and photoperiodic influences early in the pupal stage. In contrast, pupal lighting conditions had no effect on the pupal duration of the autumnal moth *E. autumnata* (Peterson & Nilssen, 1996). Since under natural conditions pupation of *M. privata* occurs inside an earthen cocoon beneath the soil surface (Elliott & Bashford, 1978), pupae in nature would presumably experience darkness and thus be more likely to enter a diapause than avert one. Finally, that *M. privata* pupae may be sensitive to photoperiodic influences should be taken into account if future studies of pupal diapause in *M. privata* are carried out under artificial lighting conditions.

Results among pupae produced under long days at 15.0°C and at 11.5°C indicate that larval development under long days did not always lead to non-diapause pupal development. Some pupae produced under long days at 15.0°C produce adults after about 290-366 days (Treatment 9 in Fig. 4.4), while some pupae produced under long days at 11.5°C produced adults after about 200-250 days (Treatment 10 in Fig. 4.4). The fact that some pupae could remain dormant for up to a year under

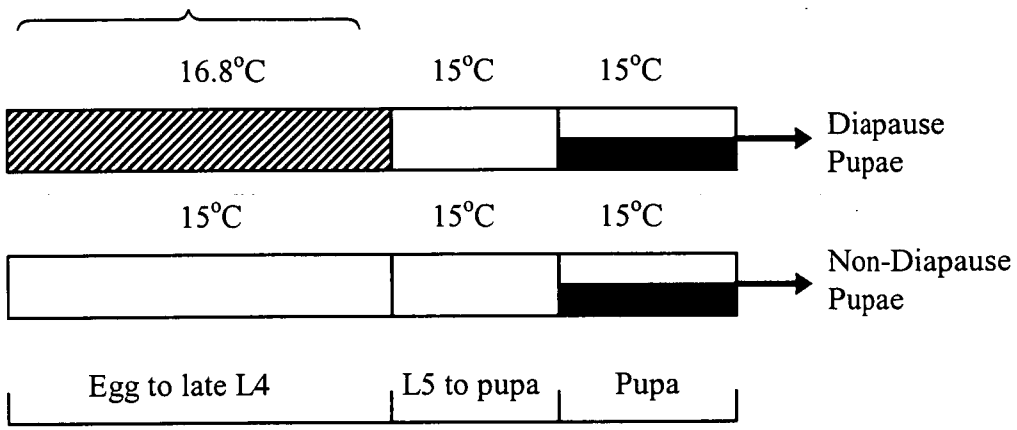
favourable conditions before producing adults suggests that a small proportion of individuals in a population might remain in diapause for extended periods even though conditions may be favourable for development. Such behaviour represents a risk-minimization strategy that may enable the species to inhabit areas where environmental conditions are unpredictable (Waldbauer, 1978; Bradshaw, 1986; Danks, 1994a). For example, pupal periods exceeding one year could provide a means for local populations to become re-established after population crashes caused by adverse environmental conditions.

Another phenomenon associated with diapause is that whereas high temperatures and constant darkness during the photosensitive period tend to promote non-diapause development in long-day species, they promote diapause induction in short-day species (Beck, 1968). Reference to Fig. 4.4 shows that the bulk of pupae produced under constant darkness at 24°C developed without a diapause. This result also suggests that *M. privata* is a long-day species. In summary, pupal diapause in *M. privata* was induced by short days experienced during larval development and was usually averted if larvae developed either under long days or under constant darkness at high temperatures. Thus, the diapause behaviour of *M. privata* appears to typify the long-day or Type I response described by Beck (1968) (see Fig. 4.1).

4.5.2 Photosensitive period

Larvae that developed to late-L4 under short days at 16.8°C produced predominantly diapause pupae despite the fact that they completed their development under long days at 15°C (Figs. 4.6 and 4.8a). This result suggested that diapause was induced before larvae reached the 5th instar. In contrast, pupae produced from a single cohort of larvae collected from the field as newly-moulted L5 on 14 March 1997 apparently took different development pathways depending on lighting conditions experienced

a) Laboratory-reared larvae:



b) Field-collected larvae:

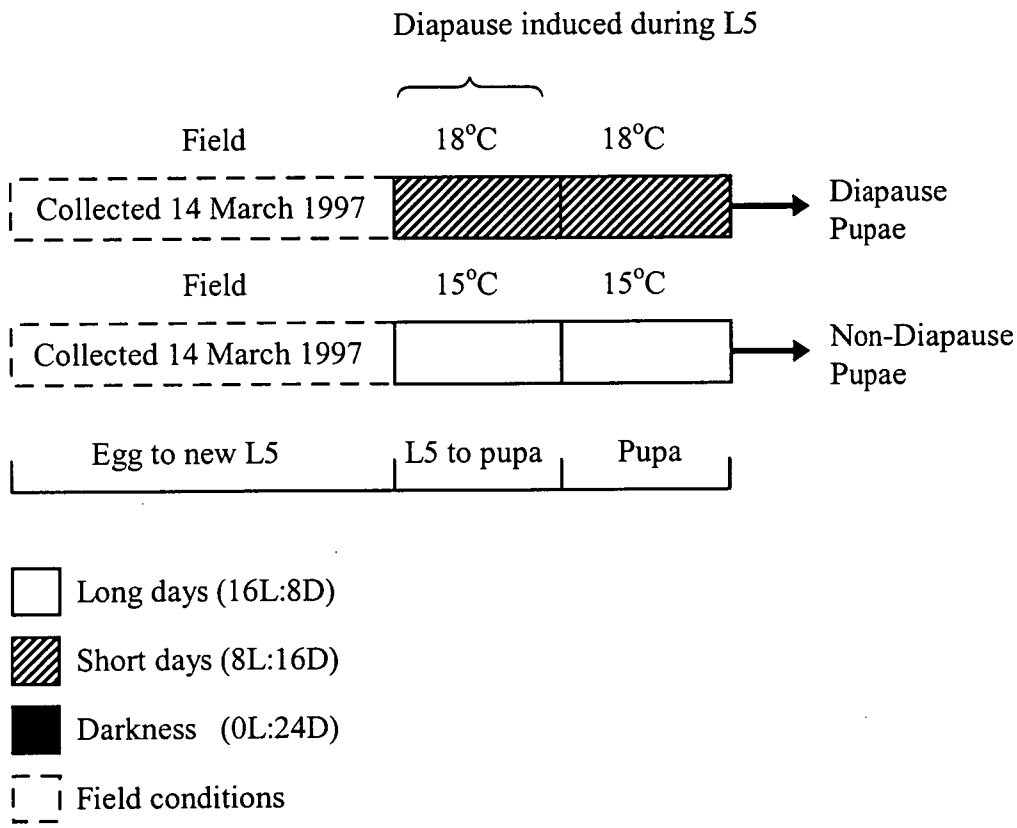


Figure 4.8 Schematic diagram showing when pupal diapause was induced during the larval stage of *M. privata*. (a) Diapause was induced by short days before the 5th instar in laboratory-reared larvae. Subsequent exposure to long days during the 5th instar did not reverse the diapause decision. (b) Diapause was induced by short days during the 5th instar (or perhaps immediately after pupation) after larvae were collected from the field as newly-moulted L5 on 14 March 1997.

during the 5th instar (pers. obs.). Larvae reared to pupation under short days (8L:16D) at 18°C produced only diapause pupae (n = 23), whereas siblings reared to pupation under long days (16L:8D) at 15°C produced only non-diapause pupae (n = 30) (data not shown). The latter result suggested that diapause was induced by short days experienced during the 5th (final) instar, as shown schematically in Fig. 4.8b. Thus, it appears that all larval stages of *M. privata* may be sensitive to diapause inducing stimuli. (NB: A temperature of 18°C is still favourable for non-diapause pupal development. It is only at temperatures above 18°C that pupal development may be interrupted by aestivation (see below).

Both scenarios in Fig. 4.8 can probably be interpreted on the basis of the three key elements of the photoperiodic response, i.e. the photoperiodic clock, the photoperiodic counter and the photosensitive period (Saunders, 1978; Takeda & Skopik, 1997):

- Larvae reared entirely under long days at 15°C did not accumulate a sufficient number of short days required for diapause induction, termed the 'required day number' (RDN) by the above authors, and hence produced predominantly non-diapause pupae.
- Larvae that developed to late-L4 under short days at 16.8°C accumulated the RDN before larvae reached the 5th instar. Once the RDN was accumulated, the induction of diapause became irreversible and larvae produced predominantly diapause pupae despite completing their developed under long days at 15°C.
- Larvae collected from the field as newly-moulted L5 on 14 March 1997 (Fig. 4.8b) had not accumulated the RDN in the field. After collection, larvae reared to pupation under short days accumulated the RDN and hence produced

diapause pupae, whereas larvae reared to pupation under long days did not accumulate the RDN and hence produced non-diapause pupae.

4.5.3 The complexity of pupal aestivation in M. privata

The results of this chapter indicate that aestivation in *M. privata* pupae is a more complicated process than previously thought. The preceding chapter found that a period of aestivation was possible late in the pupal stage (by unpigmented pharate adults) which permitted adult emergence to be delayed at high temperatures (>18°C) (Fig. 3.25). That aestivation response was termed 'late-pupal' aestivation (Section 3.5.4). However, results in this chapter found that a period of aestivation was also possible early in the pupal stage, before the first visible sign of pupal development (shifted eye spots), which permitted the start of pupal development to be delayed (Fig. 4.7). Henceforth for simplicity, the aestivation response discovered in this chapter is referred to as 'early-pupal' aestivation in order to distinguish it from late-pupal aestivation. Thus, unlike the pupal diapause of *M. privata*, which is only possible before adult differentiation begins, aestivation appears to be possible before adult differentiation begins as well as late in the pupal stage when adult differentiation is almost complete. Given this situation, four aestivation scenarios are considered possible in *M. privata* pupae. These four aestivation scenarios are shown schematically in Fig. 4.9 and described below:

a) No aestivation: If diapause ends when soil temperatures are favourable for pupal development (from 5° to 18°C) and temperatures remain favourable, pupae may develop to adult eclosion without further interruption (Fig. 4.9a).

b) Early-pupal aestivation: If diapause ends in summer when soil temperatures are high (>18°C), pupae can delay the *start* of post-diapause development (i.e. stage-1 of adult differentiation) by aestivating (Fig. 4.9b).

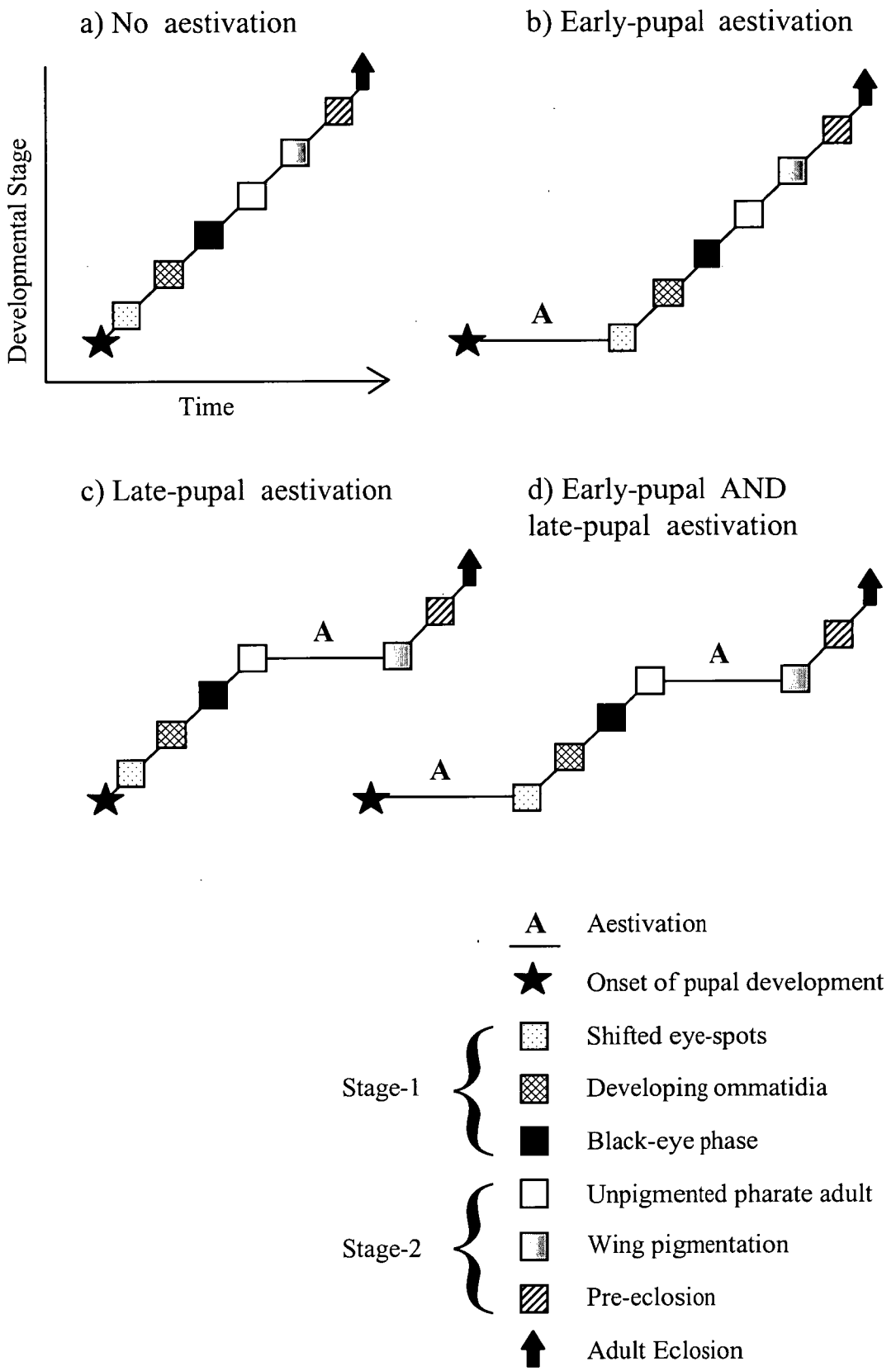


Figure 4.9 Four aestivation scenarios are considered possible in non-diapause and post-diapause pupae of *M. privata*: a) none, b) early-pupal aestivation, c) late-pupal aestivation, d) both early-pupal and late-pupal aestivation. It is proposed that early-pupal aestivation occurs immediately after the onset of pupal development.

c) Late-pupal aestivation: If diapause ends in summer when soil temperatures are temporarily favourable for pupal development (5°-18°C) but later rise above 18°C, pupae may initiate development immediately after diapause ends but then delay the *end* of post-diapause development (i.e. stage-2 of adult differentiation), also by aestivating (Fig. 4.9c).

d) Early-pupal AND Late-pupal aestivation: This is a combination of scenarios *b* and *c* above. If diapause ends during summer when soil temperatures are above 18°C, pupae can delay the *start* of post-diapause development by aestivating. Hypothetically, a temporary fall in temperature below the aestivation threshold could initiate pupal development but if temperatures rose back above 18°C, pupae could aestivate again as unpigmented pharate adults, thereby delaying the end of post-diapause development as well (Fig. 4.9d).

Scenarios *a*, *b* and *c* have been observed in this study, whereas scenario *d* has not been observed and therefore remains hypothetical. For simplicity, it is proposed that early- and late-pupal aestivation are both initiated above the same threshold temperature of 18°C.

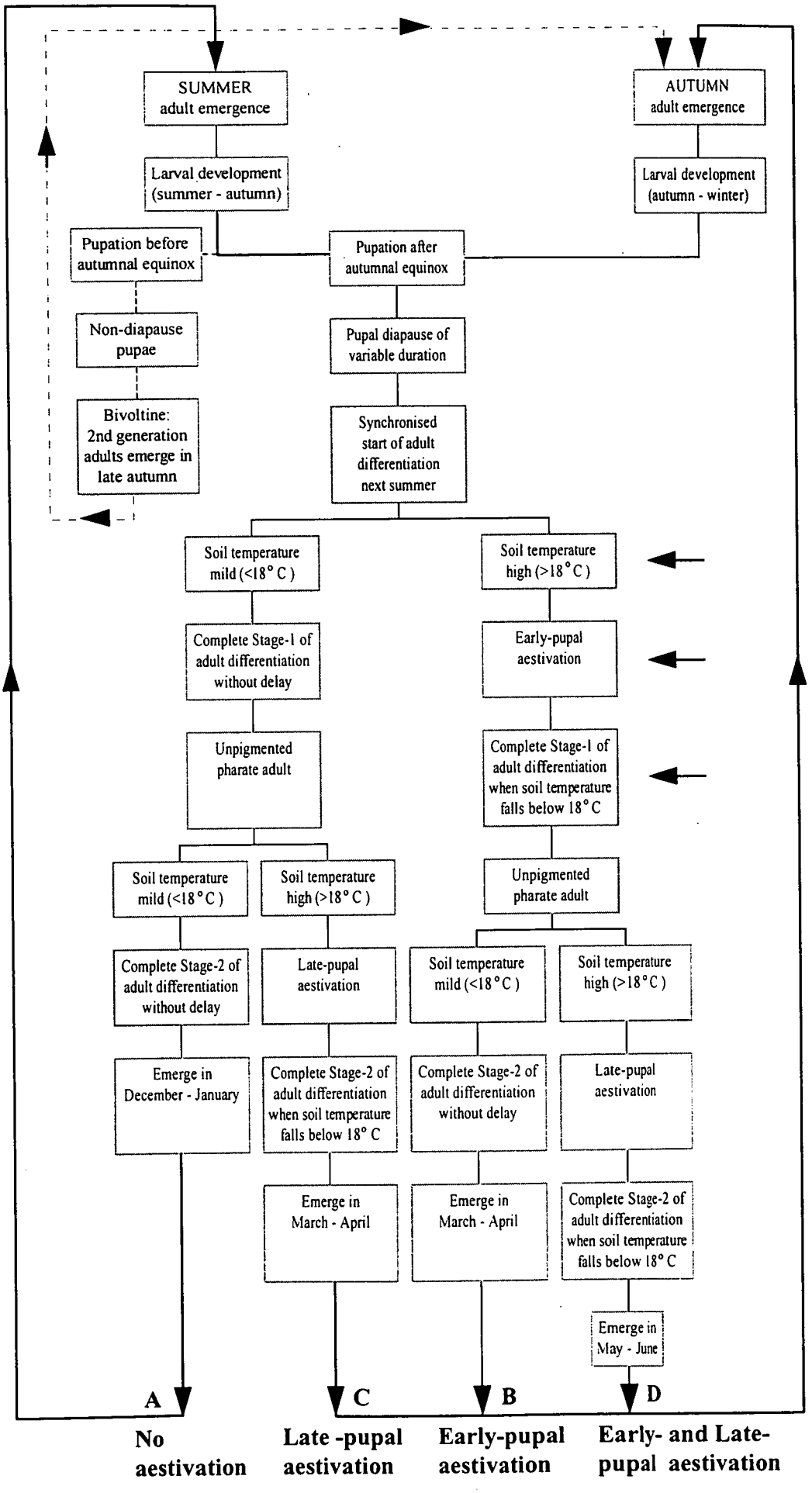
In the results, it was suggested that adults that emerged after 90-100 days from pupae produced at 21° and 24°C were from non-diapause pupae that had remained quiescent via a period of aestivation in direct response to high temperatures (Fig. 4.7). However, an alternative explanation for the results in Fig. 4.7 is suggested by the work of Cullen and Browning (1978). Their study found that *H. punctigera* pupae produced under a range of daylengths at 19°C exhibited a higher incidence of diapause when left at 19°C than if pupae were placed at 28°C. In other words, high temperatures stimulated pupal development of *H. punctigera*. Thus, pupal diapause in *M. privata* could have been induced at 21° and 24°C, but been terminated by the

month-long period that pupae spent at those temperatures before placement at 15°C. This hypothesis was later rejected when six pupae known to have entered a diapause remained in diapause at 15°C even after an attempt was made to break diapause by placing pupae at 25°C for 34 days (data not shown). It was thus concluded that adults that emerged 90-100 days after pupation at 21° and 24°C were from non-diapause pupae that had remained in a state of early-pupal aestivation (Fig. 4.9b) for exactly the times spent at those two high temperatures before placement at 15°C. This seems especially likely since (a) both temperatures are above the pupal aestivation threshold of 18°C, (b) pupae appear to have initiated development immediately after they were placed at 15°C, and (c) high temperatures tend to reduce the incidence of pupal diapause in long-day species (Beck, 1968). However, in relation to the last-mentioned point, this study has found that whilst high temperatures may avert diapause in long-day species, they may also induce a period of aestivation capable of interrupting non-diapause development. Aestivation at high temperatures could thus be mistaken for diapause in some long-day species.

Finally, the results of this chapter make it necessary to again modify the flow chart of factors influencing the phenology of *M. privata*. Specifically, it is necessary to add additional steps which allow stage-1 of adult differentiation to be delayed by a period of aestivation in direct response to high soil temperatures. Figure 4.10 shows the flow chart with these extra steps and also illustrates how the four aestivation scenarios proposed in Fig. 4.9 might influence the phenology of adult *M. privata* and lead to adult emergence from December to June in different areas of Tasmania. Such complex interactions between diapause and quiescence have been reported previously for the Australian plague locust *C. terminifera* (Wardhaugh, 1986).

Caption for facing page:

Figure 4.10 Revised flow chart of factors influencing the phenology of *M. privata* in Tasmania. The three small horizontal arrows in the right of the figure point to steps added which allow for stage-1 of adult differentiation to be delayed by a period of early-pupal aestivation in direct response to temperatures above 18°C. Late-pupal aestivation may occur in the unpigmented pharate adult and permit stage-2 of adult differentiation to be delayed as well. The four aestivation scenarios of Fig. 4.9 describe the pathways in this figure, as indicated at the bottom of the figure. The degree-day requirements for the various developmental stages have been omitted.


SUMMER adult emergence
AUTUMN adult emergence
Larval development (summer - autumn)
Larval development (autumn - winter)
Pupation before autumnal equinox
Pupation after autumnal equinox
Non-diapause pupae
Pupal diapause of variable duration
Bivoltine: 2nd generation adults emerge in late autumn
Synchronised start of adult differentiation next summer
Soil temperature mild (<18° C)
Soil temperature high (>18° C)
Complete Stage-1 of adult differentiation without delay
Early-pupal aestivation
Unpigmented pharate adult
Complete Stage-1 of adult differentiation when soil temperature falls below 18° C
Soil temperature mild (<18° C)
Soil temperature high (>18° C)
Unpigmented pharate adult
Complete Stage-2 of adult differentiation without delay
Late-pupal aestivation
Soil temperature mild (<18° C)
Soil temperature high (>18° C)
Emerge in December - January
Complete Stage-2 of adult differentiation when soil temperature falls below 18° C
Complete Stage-2 of adult differentiation without delay
Late-pupal aestivation
Emerge in March - April
Emerge in March - April
Complete Stage-2 of adult differentiation when soil temperature falls below 18° C
Emerge in May - June
A
C
B
D
No aestivation
Late -pupal aestivation
Early-pupal aestivation
Early- and Late-pupal aestivation

5. SUMMER PHENOLOGY IN TASMANIA

5.1 INTRODUCTION

Two integral parts of insect pest management are biological information, e.g. phenology of insect pest life-cycles, and technical information, such as how to reduce the size of pest populations in order to prevent economically significant damage (Pedigo, 1989). Technical information already exists which allows plantation managers to easily suppress large populations of *M. privata* should the need arise. Tree damage is readily prevented by direct application of insecticides to larvae early in the season when the bulk of the larval population is still in early instars (L1-L3) (Neumann & Collett, 1997). However, although *M. privata* can be easily controlled once detected, a major problem associated with managing *M. privata* is initial detection of populations (Farrow *et al.*, 1994; Floyd *et al.*, 1994).

As noted previously in the introduction of Chapter 2, detection of non-damaging, early life-history stages of *M. privata* is difficult because outbreaks are sporadic and sometimes localised, there is little detailed phenological information on the species and in Tasmania the phenology of *M. privata* varies with altitude (Elliott & Bashford, 1978; de Little, 1981). However, a recent outbreak (1993-94 summer) of *M. privata* in NW Tasmania occurred despite the fact that the population was detected early in the season. This incident revealed that further problems, such as how to sample the population and assess the pest risk to an area, may be encountered even after populations are detected. Hence, whilst the phenological information in previous chapters may facilitate the detection of early-season activity in different areas, more information on the biology and behaviour of early life-history stages of *M. privata* is required to improve current methods of surveying and monitoring *M. privata* populations.

Moving away from such management problems, oviposition behaviour of female insects is hypothesised to be an important factor determining whether herbivorous insect species have 'latent' or 'eruptive' population dynamics (Price *et al.*, 1990). Latent species are defined as those which remain at relatively stable population densities, varying between one or two orders of magnitude, and do not become pests, whereas eruptive species are defined as those which are able to dramatically and rapidly increase their population density by over three to five orders of magnitude, and may become pests (see Price *et al.*, 1990). Generally, it is proposed that females of latent species evaluate host plant quality as a resource for larvae and select the best oviposition sites available, whereas females of eruptive species lack the ability to evaluate host plant quality and are indiscriminate in their oviposition (Price *et al.*, 1990; Price, 1994). Therefore, a better understanding of *M. privata* oviposition behaviour in particular may help explain its eruptive population dynamics. On the other hand, eruptive population dynamics may instead reflect that *M. privata* is an *r*-selected species adapted to living in r-selecting environments which typically are unpredictable in time or ephemeral (see Begon *et al.* (1986) p. 522). This differs from stable population dynamics of *K*-selected species living in *K*-selecting environments which are relatively constant or predictably seasonal in time (Begon *et al.*, 1986).

This chapter presents the results of a field study designed to collect empirical data on early-season activity and oviposition behaviour of *M. privata* at high altitudes in Tasmania. The objectives of the study were to: (i) collect further evidence of early adult phenology at high altitudes in Tasmania; (ii) gain a better understanding of where eggs are likely to be found on host trees during surveillance operations; (iii) compare the oviposition behaviour of *M. privata* with that expected

of an eruptive species; and (iv) demonstrate the potential impact of summer outbreaks of *M. privata* in high-altitude plantations in Tasmania.

5.2 METHODS

5.2.1 Study site and previous infestation history

Surrey Hills (41° 21'S, 145° 45'E) is a privately owned tree farm situated on a 625 km^2 undulating basalt plateau in NW Tasmania. The soils are brown ferrosols, are fertile and have good structure and drainage properties (G. Holz, unpubl. data). The study site was a 175 ha plantation established in September 1991, hereafter called the *Wages Rd* plantation. One third of the plantation had been extensively damaged by an extreme outbreak of *M. privata* between December 1993 and April 1994 and the plantation looked threatened again in December 1994, when a large population of adult moths began emerging (A. Clarke, pers. comm.). Tree spacing was 3m x 3m, giving 1194 trees/ha. Hence, approximately 70 000 trees were damaged in the 1993-94 outbreak. Site elevation is between 600 and 640 m ASL. Mean daily minimum and maximum temperatures, based on the nearest meteorological station of Waratah (41° 27'S, 145° 32'E) (612 m ASL, 18km SW of *Wages Rd*) are 6.3 and 17.6°C during January and 0.8 and 7.2°C during July. Mean annual rainfall is 2204 mm falling predominantly in winter (Australian Bureau of Meteorology, 1998).

The level of insect damage to trees in the *Wages Rd* plantation was visually assessed in April 1994 (i.e. immediately after the 1993-94 outbreak, but six months before the present study commenced) by associates and subjectively divided into three damage classes: hotspot, heavily defoliated and undamaged (S. Paterson and V. Patel, pers. comm.) (Fig. 5.1). General descriptions of each damage class are as follows:

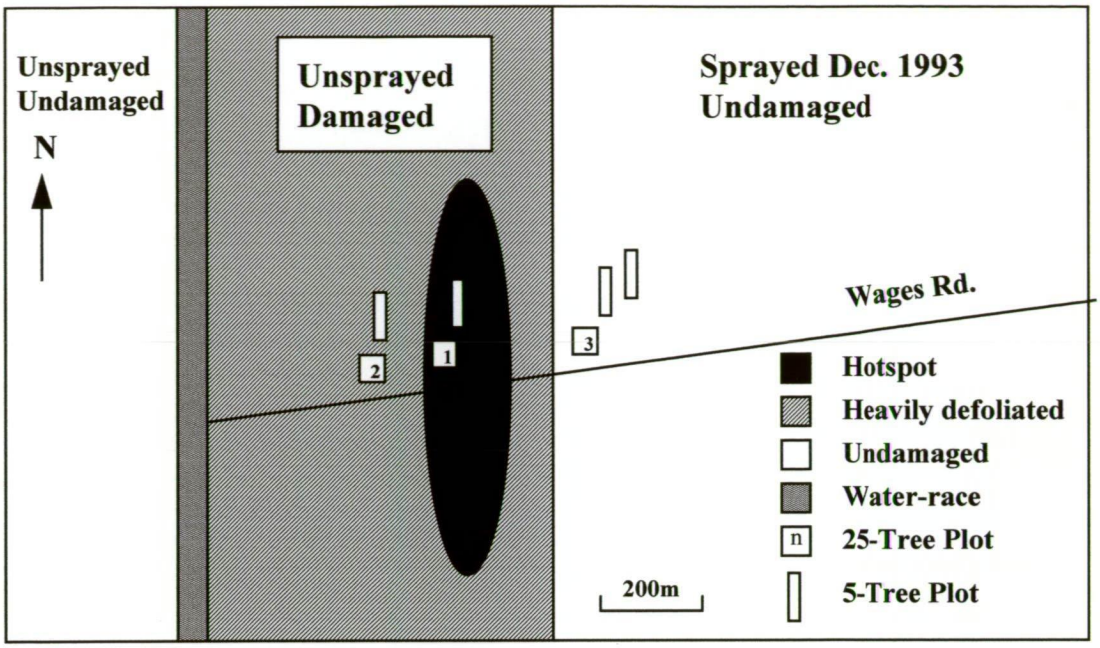


Figure 5.1 Schematic diagram of the pattern of damage by *M. privata* at *Wages Rd.*
Notes:

- The hatched area represents a heavily defoliated area sandwiched between a water-race to the west and the sprayed area to the right.
- Small squares numbered 1-3 indicate 25-tree study plots in the three damage classes used to assess egg batch positions on host trees and host-plant shoot phenology.
- The four small rectangles without numbers indicate 5-tree study plots in the three damage classes used to assess mean batch size.

Figure 5.2 One of two bays of *Eucalyptus nitens* trees in the hotspot area at *Wages Rd.* The hotspot occurred around the large windrow in the right of the picture (see arrow).

1) Hotspot area– Trees in a 120 m wide strip running north-south through the plantation were almost totally defoliated. That strip was designated the 'hotspot' of *M. privata* activity and consisted of two bays of trees, one bay on each side of a large windrow (Fig. 5.2). Windrows are graded mounds of old vegetation that are not

removed from the site during preparation for tree-planting. A bay is a block of trees between two windrows. Most bays had about 20 rows of trees.

2) Heavily defoliated area– A large section of the plantation, predominantly west of the hotspot, had also been heavily defoliated, but not as severely as the hotspot area. This section was confined to 8 bays (ca. 160 rows spread over 500 m) between the hotspot and a water-race running through the western end of the plantation. This area and the hotspot comprise the foreground section of the plantation in Fig. 1.2 where the red-brown soil colour is visible through the tree canopies.

3) Undamaged area– The remaining 17 bays of trees (about 340 rows over 1000 m) to the east of the hotspot were undamaged because they had been aerially sprayed with insecticide in December 1993 to protect a fertiliser trial within that area from insect damage. Also largely undamaged was the plantation's western end on the other side of the water-race.

When the field study began in December 1994, many trees in the hotspot and heavily defoliated area had vigorous new juvenile shoots on lateral branches, whereas growth of trees in the undamaged area was most vigorous in the upper canopy, where many trees had started to produce adult foliage (pers. obs.). The lower canopies of undamaged trees still carried mainly juvenile foliage, but it was mostly older foliage from previous growing seasons.

5.2.2 Adult phenology

On six occasions between 15th and 29th December 1994, adult moths were caught in the hotspot area at *Wages Rd*, primarily as evidence that adults emerged during the December-January period as previously reported by de Little (1981). On the first 5 occasions, a sweepnet was used to catch flying moths, active in the early hours of the evening. However, on the 29th December 1994, moths were inactive (presumably due to cold) and were collected by shaking the ends of lateral branches placed inside

the sweepnet. Each collection period lasted 45 minutes between 8:30 and 9:15pm. Captured moths were counted and sexed (see Elliott & Bashford, 1978). The following summer (1995-96), a black-light trap was used to capture moths at *Wages Rd* instead of a sweepnet. The light trap was set on six nights between 27th November 1995 and 20th February 1996 in the same area of the hotspot where moths were caught by sweepnet in December 1994. Captured moths were counted and sexed as before.

5.2.3 Oviposition sites on host trees

The position of oviposition sites on host trees was investigated on trees in each category of damage at *Wages Rd* (Fig. 5.1). Each study plot consisted of five rows of five trees, although seven and five trees were dead in the hotspot and heavily defoliated plots respectively. The sample unit was a lateral shoot of juvenile foliage from the initiation point of current season's growth. Ten sample shoots were selected at random from each aspect (N, S, E, W) in the tree canopy and examined for eggs. When fewer than 10 shoots were available for sampling on any aspect of a particular tree, that aspect on the tree was excluded. Egg batch positions were recorded as the number of leaf pairs behind the tender apical bud, classified as A_0, and in front of the hard overwintering bud, classified as B_0. Thus, a position of A_1 B_4 indicated an egg batch one leaf pair behind the apical bud and four new leaf pairs in front of the overwintering bud, while A_2 B_3 indicated an egg batch situated two leaf pairs behind the apical bud and three new leaf pairs in front of the overwintering bud (Fig. 5.3). Although these examples represent different egg batch positions, they both indicate a sample shoot with four newly opened leaf pairs, calculated from $x + y - 1$, where x and y refer to A_x B_y. This method not only recorded egg batch position, but also provided an indication of host-plant shoot phenology during *M. privata*'s oviposition period.

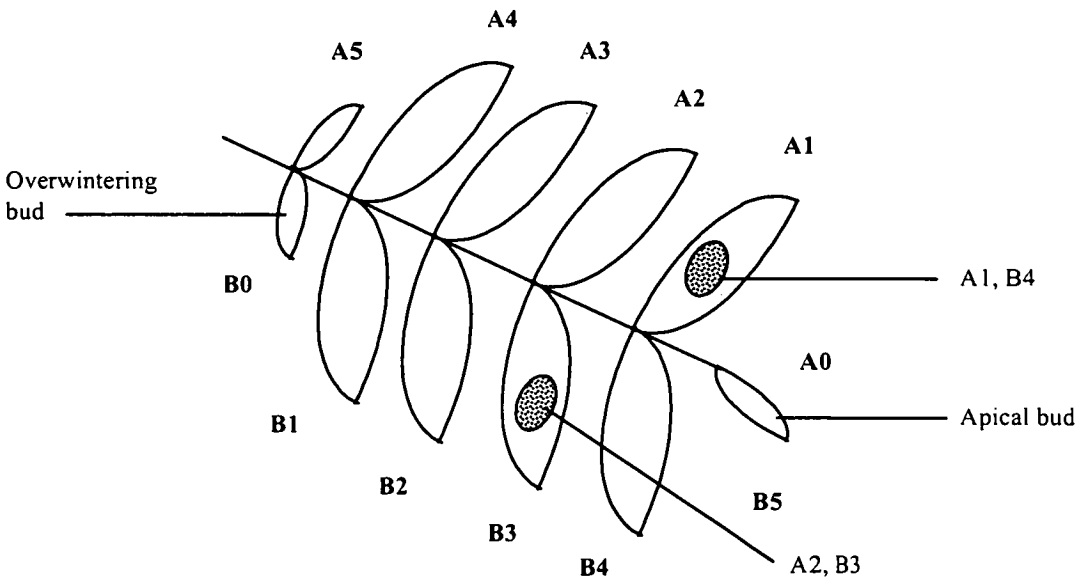


Figure 5.3 Schematic diagram of a sample shoot with two egg batches in different positions. The numbers following the letter A refer to the number of leaf pairs before the apical bud, A_0. The numbers following the letter B refer to the number of leaf pairs after the overwintering bud, B_0.

The proportion of egg batches in each canopy aspect was assessed in each plot by Chi-Square analysis, testing the null hypothesis that eggs were evenly distributed among the four aspects (i.e. P=0.25). A 4x3 contingency table tested whether the proportion of batches in each aspect varied from plot to plot. Finally, frequency distributions made from the leaf-class data were used to estimate the most common egg batch position on sample shoots and to determine the number of new leaf pairs on an average sample shoot.

The mean number of eggs per batch (hereafter termed batch size) was determined with a one-off sample of egg batches collected from each damage type on 20th December 1994. From the eastern half of the canopy of a tree, all leaves with egg batches were removed up to a height of 2 m. Five trees were sampled in the hotspot area, five trees in the heavily defoliated area and ten trees in the undamaged area, where egg batches were less common. These 20 trees were situated outside the three 25-tree study plots used above (see Fig. 5.1). Eggs were collected from the

eastern half of the canopy after preliminary surveys found that eggs tended to be found in greater numbers on that side of the canopy (see results). Number of leaves with eggs, number of egg batches and the size of each egg batch were determined in each of the three samples. One-way ANOVA was used to test whether mean batch size varied in the different damage classes, while leaf number and batch number in the three samples provided general information on whether females had a tendency to lay more eggs in previously defoliated or undamaged areas. The number of new egg batches present on the same trees was counted after 7 days (27 December 1994) and the proportion of new to old batches used to indicate whether the distribution of egg batches remained constant or changed with time.

5.2.4 The impact of severe defoliation at Wages Rd

The impact of severe defoliation was assessed in a block of 1606 trees within the hotspot area at *Wages Rd* between 27 December 1994 and 17 March 1995, approximately 9-11 months after severe defoliation the previous growing season. The block consisted of 21 rows of between 71 and 93 trees. Each tree in the block was visually assessed and given a subjective recovery rating from 1-5, where 1 indicated a dead tree with no visible shoots, 2 indicated a tree with only a few tiny shoots and 3-5 indicated recovery ratings of poor, fair and good respectively based on the density of leaves. The number of trees in each category was assessed to determine the impact of severe defoliation by *M. privata* at this high-altitude site.

5.3 RESULTS

5.3.1 Adult phenology

The number and sex ratio of *M. privata* adults caught in the hotspot area at *Wages Rd* by sweepnet and by light trap are presented in Table 5.1. In December 1994, a total of 721 moths was caught by sweepnet over six evenings, with a mean of 120.2 ± 13.7 (SE) moths being caught each night (Table 5.1). The following summer, only 11

Table 5.1 The number and sex ratio of *Mnesampela privata* adults caught by sweepnet and black-light trap at the *Wages Rd Eucalyptus nitens* plantation at *Surrey Hills* in NW Tasmania.

Method	Date	Male	Female	Total	Min T [a] (°C)	Sex Ratio M : F
Sweepnet	15.12.94	145	4	149	7.2	36.3 : 1
	18.12.94	52	6	58	8.6	8.7 : 1
	21.12.94	114	0	114	4.5	114.0 : 0
	24.12.94	142	6	148	5.8	23.7 : 1
	27.12.94	125	6	131	5.4	20.8 : 1
	29.12.94 [b]	110	11	121	6.6 [b]	10.0 : 1
94-95 Total		688	33	721		20.8 : 1
Mean ± SE		114.7 ± 13.8	5.5 ± 1.5	120.2 ± 13.7		
Light trap	27.11.95	0	0	0	6.4	0 : 0
	28.11.95	1	0	1	0.6	1 : 0
	12.12.95	1	0	1	5.6	1 : 0
	03.01.96	2	0	2	8.8	2 : 0
	16.01.96	3	0	3	2.9	3 : 0
	14.02.96	4	0	4	8.7	4 : 0
95-96 Total		11	0	11		11 : 0
Mean ± SE		1.8 ± 0.6	0	1.8 ± 0.6		

[a] Refers to the minimum temperature recorded on the trapping night, not the temperature at the time when moths were captured. Temperatures were recorded approx. 5km from the *Wages Rd* plantation, and kindly provided by G. Holz, North Eucalypt Technologies.
[b] No flight activity was observed on 29.12.94. This is surprising since moths were caught on other occasions, e.g. 21.12.94 when the minimum recorded temperature was below 6.6°C. Despite this anomaly, a low temperature at the actual trapping time is still thought to be responsible for the sedentary behaviour on the 29th.

moths were caught by light trap over six whole nights, with an average of 1.8 ± 0.6 moths caught per night (Table 5.1). Sex ratios at *Wages Rd* were heavily biased towards males, with an overall ratio of 20.8 males captured for each female in December 1994, and 11 males only captured the following season by light trap (Table 5.1).

5.3.2 Oviposition sites on host trees

The proportion of egg batches present on each canopy aspect is presented in Figure 5.4. Egg batches were not evenly distributed among the four canopy aspects in the hotspot ($X^2_3 = 9.17$, $p=0.03$), heavily defoliated ($X^2_3 = 48.40$, $p<0.001$) and undamaged ($X^2_3 = 11.41$, $p<0.01$) plots and the proportion of batches laid in

different canopy aspects did not vary from plot to plot ($X^2_6 = 9.24$, p=0.16). Generally, eggs were present in greater numbers on the eastern aspect of the canopy in all plots, with 71% and 78% of egg batches in the hotspot and heavily defoliated plots respectively being collected from the SE half of tree canopies and 73% of batches in the undamaged plot being collected from the NE half of tree canopies (Fig. 5.4). The mean number of batches per ten sample shoots is presented in Table 5.2. The table shows that the number of batches per ten shoots in the heavily defoliated plot was more than double the number present in the other two plots.

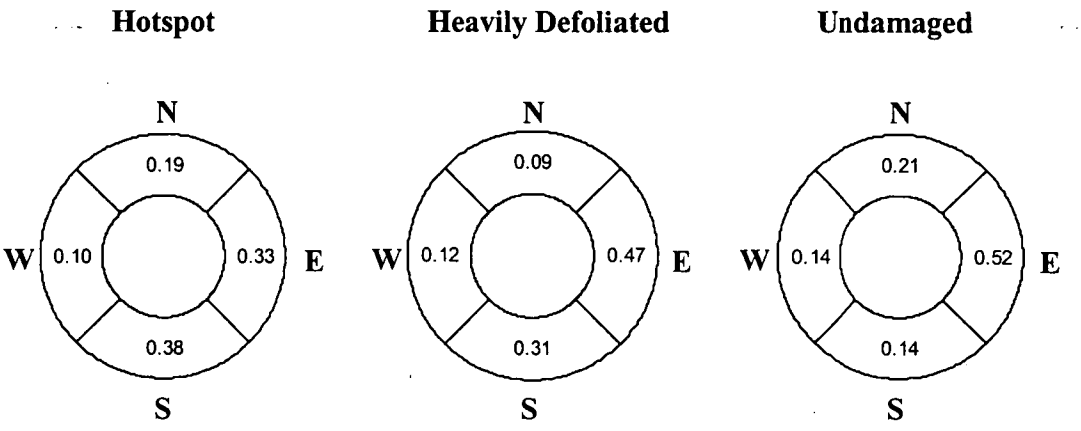


Figure 5.4 The proportion of *M. privata* egg batches on each canopy aspect in the lower 2 m of the canopies of juvenile *E. nitens* trees.

Table 5.2 The average number of *M. privata* egg batches present on ten sample shoots of juvenile *E. nitens* foliage. Batches were counted on the shoots, but not collected.

	Hotspot			Heavily defoliated			Undamaged		
Aspect	No. of shoots	No. of batches	Batches per 10 shoots	No. of shoots	No. of batches	Batches per 10 shoots	No. of shoots [a]	No. of batches	Batches per 10 shoots
N	150	9	0.60	170	12	0.71	80	6	0.75
E	170	16	0.94	170	60	3.53	120	15	1.25
S	150	18	1.20	150	40	2.67	100	4	0.40
W	150	5	0.33	170	15	0.88	100	4	0.40
Total	620	48	0.77	660	127	1.92	400	29	0.73

[a] Dividing the number of shoots by 10 indicates the number of trees with at least 10 sample shoots in that particular canopy aspect. Number of sample shoots in the undamaged area was low because many trees produced few or no juvenile shoots suitable for sampling.

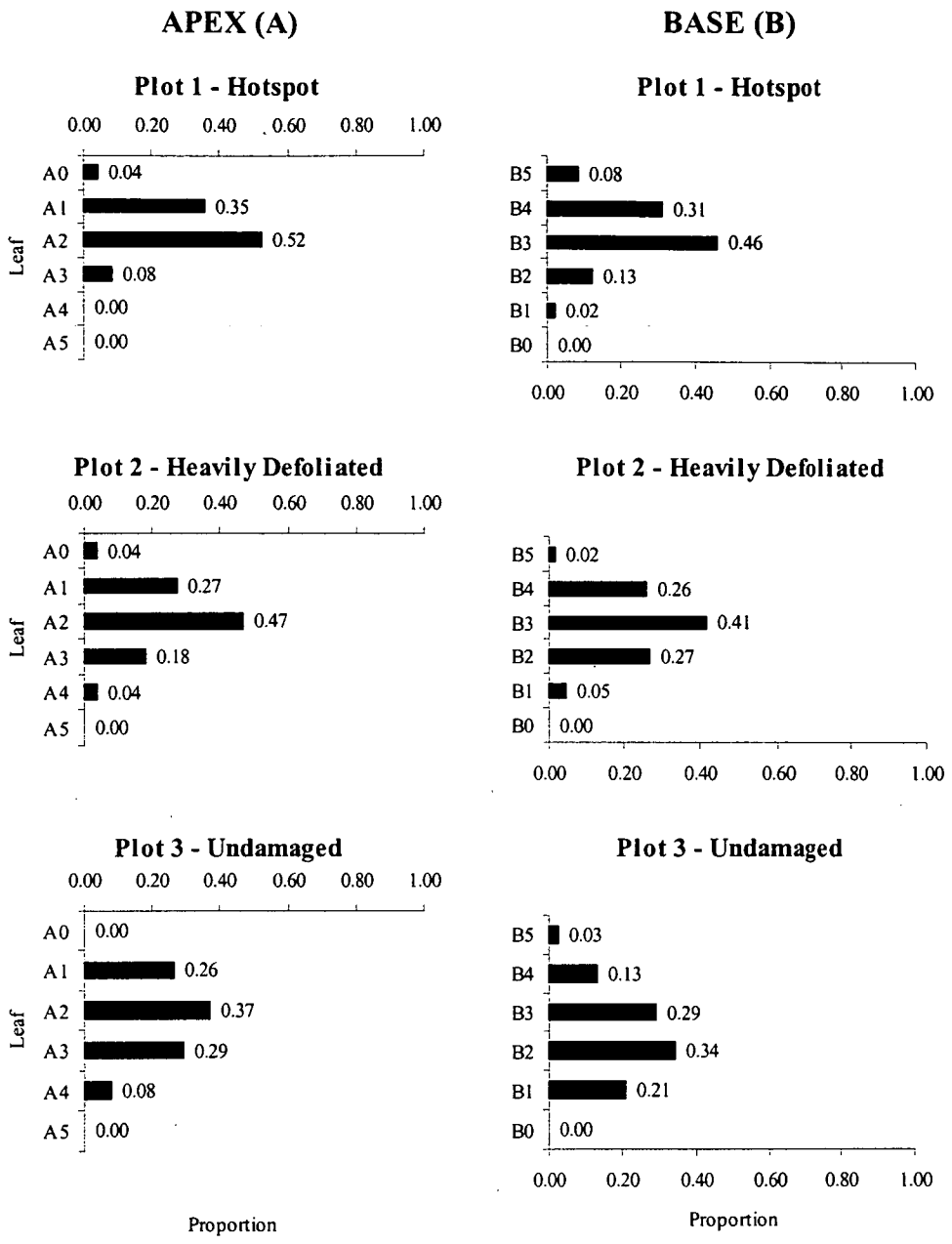


Figure 5.5 Position of *Mnesampela privata* egg batches on sample shoots of *Eucalyptus nitens* in plots of trees that had suffered different levels of defoliation the previous season. The left-hand and right-hand sides of the figure show egg batch position in relation to the number of opened leaf pairs behind the apical bud (A_0) and in front of the overwintering bud (B_0) respectively.

The position of oviposition sites on sample shoots is presented in Figure 5.5. The highest proportion of egg batches was found on A_2, the second leaf pair behind an apical bud. This pattern was found in all plots, with 52, 47 and 37% of batches being found on A2 in plots 1-3 respectively (Fig. 5.5). This position was usually associated with B3, the third leaf pair in front of an overwintering bud (Fig. 5.5),

although in the undamaged area batches were slightly more common at position B2 than B3. On the basis of the frequency distributions, an 'average' sample shoot had 2+3-1= 4 newly opened leaf pairs between the overwintering and apical buds (Figs. 5.6 and 5.7).

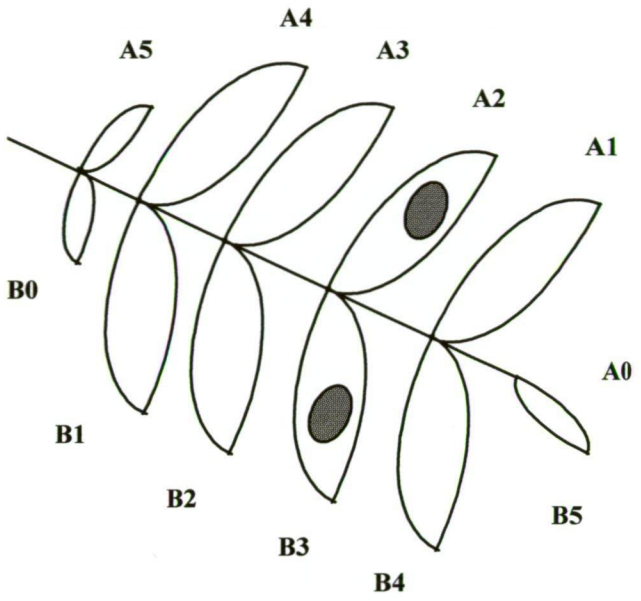


Figure 5.6 A typical *Eucalyptus nitens* sample shoot in the hotspot and damaged areas of the *Wages Rd* plantation during mid-December 1994, based on Fig. 5.5. Four new leaf pairs had opened between the overwintering bud (B_0) and the apical bud (A_0). Stippled circles on leaves A_2 B_3 represent the 'average' position of egg batches on sampled shoots.

Figure 5.7 *Mnesampela privata* female ovipositing on a juvenile *E. nitens* shoot in the undamaged area in the early hours of the evening. Note the four new leaf pairs between the apical bud and the yellowish leaf from the overwintering bud. The moth is ovipositing on a leaf that would be classified as A_1 B_4.

The number of egg batches, and the total number of eggs collected from the eastern side of tree canopies were highest in the hotspot plot, intermediate in the heavily defoliated plot and lowest in the plot of undamaged trees (Table 5.3). However, the mean batch sizes of 68.5 ± 3.5, 70.0 ± 5.1 and 56.8 ± 4.7 eggs respectively in plots 1-3 were not significantly different (ANOVA: $F_{2,421} = 1.42$, $p = 0.24$), indicating that different egg densities in different areas of the plantation were due to differences in the number rather than the size of egg batches. The mean batch size for all batches sampled was 67.1 ± 2.6 (n= 424). The distribution of egg batches varied with time since the proportion of egg batches present on sample trees in the hotspot area almost halved between 20 and 27 Dec, while the proportion of egg batches present on sample trees in the heavily defoliated and undamaged areas almost doubled over the same period (Table 5.3).

Table 5.3 The number of *Mnesampela privata* eggs laid in the eastern half of the canopies of juvenile *Eucalyptus nitens* trees sampled on 20 December 1994 and then 7 days later.

Item	Hotspot 5 trees	Heavily Defoliated 5 trees	Undamaged 10 trees	Total
20th December 1994				
Total egg batches collected	264	97	63	424
Egg batches per tree (mean)	52.8	19.4	6.3	21.2
Proportion of total egg batches (%)	62	23	15	100
Total leaves with egg batches	154	74	46	273
Total egg number	18 083	6 786	3 579	28 448
Batch size (Mean ± SE)	**68.5 ± 3.5**	**70.0 ± 5.1**	**56.8 ± 4.7**	**67.1 ± 2.6**
Range	1 - 289	8 - 281	2 - 189	1 - 289
27th December 1994				
New egg batches [a]	121	123	86	330
Egg batches per tree (mean)	24.2	24.6	8.6	16.5
Proportion of total egg batches (%)	32	39	29	100
Total leaves with new egg batches	80	97	71	248

[a] Newly laid egg batches were not removed from the tree to determine batch size.

5.3.3 The impact of severe defoliation at Wages Rd

The proportion of trees in each recovery class is presented in Table 5.4. The overall mortality rate among the 1606 trees was 32%, with a further 7% of trees having only tiny shoots. The mortality rate within a row ranged from 72% immediately next to the windrow to just 3% in row 21 which was around 60 m away from the windrow. Generally, the proportion of dead trees within a row declined as distance from the windrow increased (regression: $F_{1,19} = 69.28$, $p < 0.001$, $r^2 = 0.78$) (Fig. 5.8). Meanwhile, the proportion of trees recovering from defoliation increased with increasing distance from the windrow (Fig. 5.8). After finding such high mortality along one side of the windrow, tree mortality was also assessed on the other side, but only in the first two rows. Tree mortality rates in rows 1 and 2 on the other side of the windrow were 70 and 54% respectively, which suggested a similar trend in tree mortality on the other side of the windrow as well. The spatial pattern of mortality among the assessed trees at *Wages Rd* is presented in Fig. 5.9. The figure clearly shows a concentration of dead trees around the central windrow.

Table 5.4 The number and proportion of *E. nitens* trees in each of five recovery classes among 1606 trees assessed in the hotspot area of the *Wages Rd* plantation.

Recovery class	Class 1 Dead	Class 2 Tiny Shoots	Class 3 Poor	Class 4 Fair	Class 5 Good	Total
Number	516	117	199	423	351	1606
Proportion (%)	32.1	7.3	12.4	26.3	21.9	100

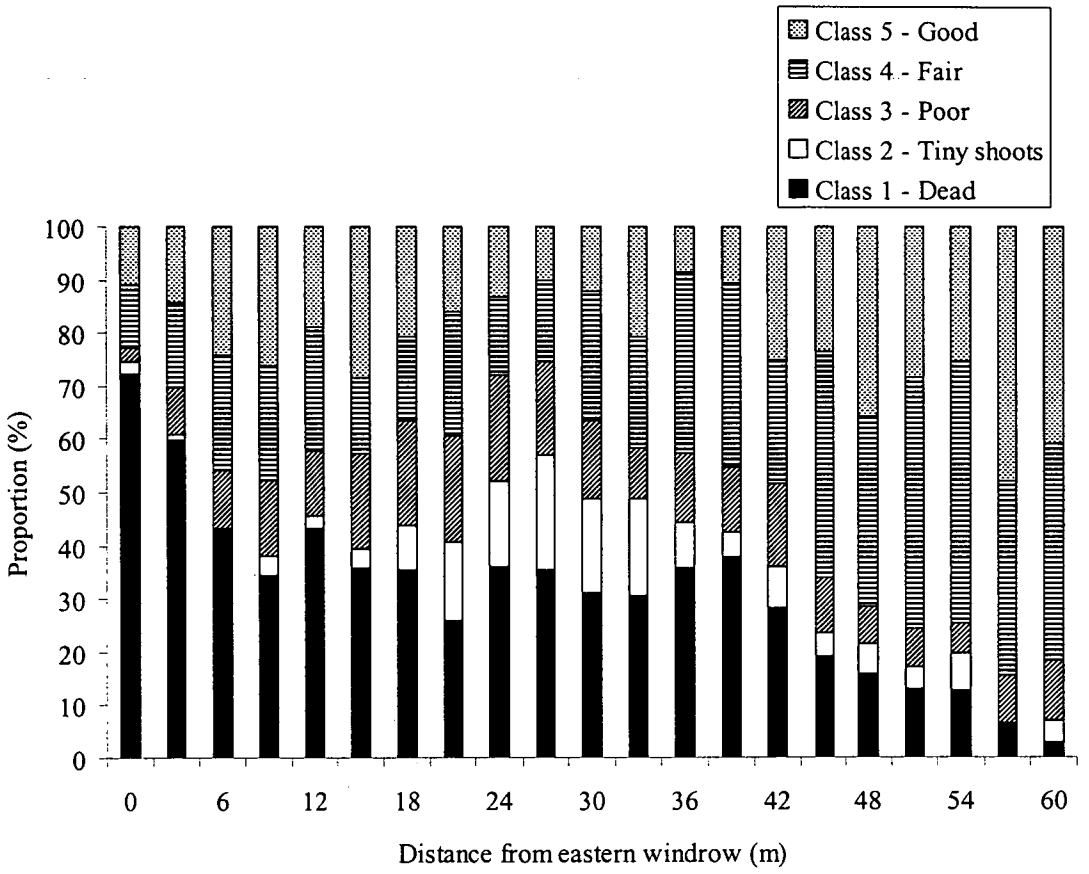


Figure 5.8 The proportion of *E. nitens* trees in each of five recovery classes in one bay of the hotspot area of the *Wages Rd* plantation. Each column represents a single row of between 71 and 93 trees.

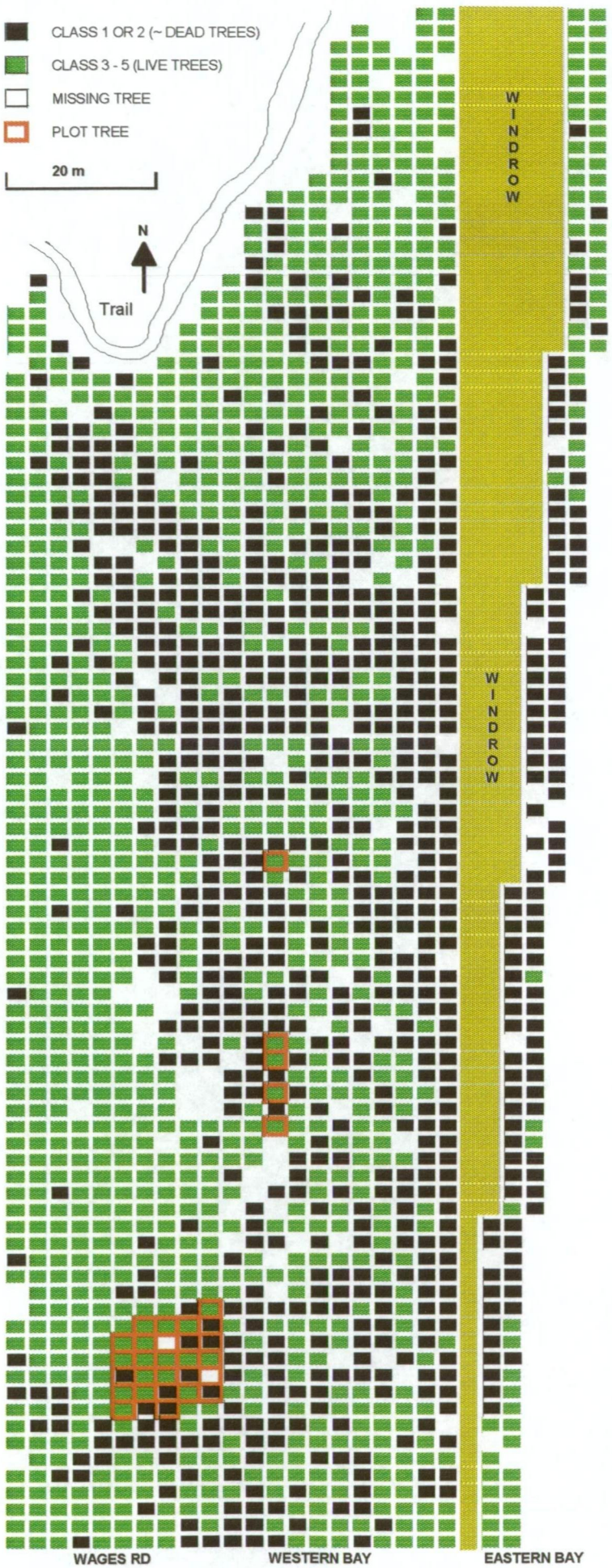


Figure 5.9 The spatial pattern of mortality among *E. nitens* trees assessed in the hotspot area of the *Wages Rd* plantation. Note the concentration of dead trees around the windrow. Red squares in the western bay indicate trees used to study oviposition patterns.

5.4 DISCUSSION

5.4.1 Adult phenology

The population density of *M. privata* was so high in the hotspot bays of the *Wages Rd* plantation that hundreds of moths were seen flying at any one time during five of the six sampling occasions. An average of 120.2 ± 13.7 (n=6) adults caught per 45 minutes of sweepnetting reflects this high population density. Adult phenology was very similar to that reported by de Little (1981) and underlines the tendency for moths to emerge during December and January at this high-altitude site in NW Tasmania. Using a light trap, de Little (1981) collected 956 moths over ten whole nights compared with 721 moths collected by sweepnet over six 45 minute periods in this study. Although these results are based on different collection methods, and should not be directly compared, they do suggest that population density may have been higher in this study than that observed by de Little (1981).

A key finding in relation to *M. privata* adult phenology was the sedentary behaviour on 29th Dec. 1994, even when moths were physically disturbed. When moths were dislodged from host-tree foliage, they simply fell to the ground or into the sweepnet during collection. This contrasted their behaviour during the day time when otherwise inactive moths usually flew away rapidly when disturbed to avoid capture. Such sedentary behaviour was observed again a year later in other high-altitude plantations (around 600 m ASL) south of Tarraleah in central Tasmania. Adult moths were unable to fly at dawn on 19th Jan. 1996, at which time a temperature of 3°C was recorded (pers. obs.). On that occasion, 217 sedentary moths were collected by hand from the ground and trees surrounding light traps set in the plantations the previous evening. This figure compared with 143 moths actually taken by the two light traps. It is assumed that low temperatures inhibited moth activity on both 29.12.94 and 19.01.96 and that moths present in the vicinity of the

light traps at Tarraleah on 19.01.96 were attracted there the previous evening and became sedentary once the temperature fell below their activity threshold. Had the traps set at Tarraleah in January 1996 been emptied several hours after dawn, perhaps moths found in the vicinity of the traps at dawn would have dispersed and hence gone un-noticed. These observations indicate that if light traps are set on very cold nights, adult moths may not be caught even if they are present in an area.

Assuming that low temperatures inhibit adult activity, adult emergence during summer at high altitudes in Tasmania may be advantageous since adult activity would coincide with the annual period of highest temperatures, thereby maximising the chance for successful survival and reproduction. Were adults to emerge in autumn at high elevations in Tasmania as they do throughout the remainder of the species distribution, they would be exposed to temperatures that would limit their activity and ability to reproduce. This hypothesis is consistent with Tammaru *et al.* (1996), who found that minimum night temperature is the main factor influencing the oviposition rate of the autumnal moth, *E. autumnata*. However, adult moths of some species adjust to low evening temperatures by shifting their daily period of activity to the hours before sunset when temperatures are warmer (Riedl, 1983). Adult moths of *M. privata* may be able to adjust their daily period of activity under similar circumstances, but this is considered unlikely since adult flight was not observed before dusk at any time during this study unless moths were disturbed.

The sex ratio of adults was consistently male-biased and could indicate a greater proportion of males in the population or be a reflection of behavioural differences between the sexes which make males more likely to be captured. Based on a more even sex ratio of 0.88:1 (m:f) among 324 pupae reared from fifth instars collected at *Surrey Hills* and Tarraleah between February and May 1996

(the pupae studied in Chapter 2), behavioural differences between the sexes seem the most likely explanation. Perhaps males were active in the air searching for virgin females, virgin females were sedentary on host trees waiting for males to find them and mated females were ovipositing on host trees. Males of many other insect species are also more likely to be caught because of their mate-searching behaviour (Thornhill & Alcock, 1983).

5.4.2 Population collapse

The small number of moths (n=11) caught by light trap at *Wages Rd* between November 1995 and February 1996 reflected a near absence of moths, since moths were readily taken by light trap during January 1996 in other high-altitude plantations by the author (as discussed above) and at *Surrey Hills* in de Little's (1981) study (956 moths caught over ten nights). de Little's (1981) study in particular shows that light traps are effective at collecting adult moths. The reason for the population collapse at *Wages Rd* remains unknown, but highlights the eruptive nature of *M. privata*'s population dynamics. The near absence of moths in the 1995-96 summer suggests that either very few larvae reached the pupal stage in the 1994-95 season, or that most pupae forming in that season did not survive through the winter months. The fact that trees at *Wages Rd* were not heavily defoliated by April 1995, despite heavy skeletonizing early in the season (pers. obs.), suggests that larval rather than pupal mortality was more important. Larval mortality due to starvation seems unlikely because, as noted above, trees at *Wages Rd* were not heavily defoliated by the end of the 1994-95 season. Perhaps the population collapse was caused by diseases spreading through dense larval populations in the second year of the outbreak (1994-95). This was not tested but warrants further study since diseases are known to affect the population dynamics of other forest lepidopteran pests (Shepherd, 1994; D'Amico & Elkinton, 1995; Roland & Kaupp, 1995).

For example, in the genus *Chlenias* (Lepidoptera: Geometridae) species attacking radiata pine were suppressed by nuclear polyhedrosis virus while species attacking *Cupressus macrocarpa* were suppressed by granulosis virus (Madden & Bashford, 1977).

5.4.3 Oviposition patterns

The large number of egg batches (n= 424) collected from just 20 trees (Table 5.3) indicated that oviposition was well underway by the end of December 1994, which agreed with de Little's (1981) findings. Eggs tended to be laid on more easterly aspects of tree canopies. Since the latitude of Tasmania (40°38' to 43°39' south) places it within the broad band of westerly winds commonly known as the 'roaring forties' (Australian Bureau of Meteorology, 1995), oviposition on easterly aspects of tree canopies may reduce the risk of eggs and unprotected early instars being dislodged from the leaf surface in adverse weather. Alternatively, eggs may have been laid on easterly aspects because moths fly against the wind (up an odour plume) when searching host trees upon which to oviposit. These results, and the fact that the most common egg batch position was two leaves behind an apical bud (Fig. 5.6), suggest that when sampling for eggs and young larvae of *M. privata* early in the season, it may be best to search the terminal leaves of lateral shoots on the side of trees facing away from the most commonly experienced wind direction. It could be argued that females oviposited predominantly on expanding young shoots because young shoots were the only shoots available after severe defoliation the previous season. However, this argument breaks down in the undamaged area, where females could have oviposited on old foliage from the previous growing season, but did not.

Mean egg batch size was relatively uniform at about 70 eggs per batch in the three sample plots, but the number of batches collected on 20 Dec. 1994 differed markedly between sites. The mean number of egg batches per tree in the hotspot

plot was almost three times that in the heavily defoliated plot and about eight times that in the undamaged plot, while the number in the heavily defoliated plot was about three times that in the undamaged plot (Table 5.3). The most likely explanation for this pattern is that the distribution of eggs simply reflected different densities of adult moths in the different areas. Although not quantified, moth density was clearly highest in the hotspot area, lowest in the undamaged areas and intermediate in the heavily defoliated area (pers. obs.). This is why so many moths could be caught by sweepnet in the hotspot area. Such a method would have been far less successful elsewhere in the plantation since dense swarms of moths did not occur outside the two hotspot bays. This implies that, at least soon after emergence, moths do not fly far. An alternative explanation for the greater abundance of eggs in the hotspot and heavily defoliated areas could be that previously defoliated trees were more attractive to ovipositing females because they had more juvenile shoots (preferred for oviposition) than undamaged trees. Trees recovering from defoliation in 1994 produced predominantly juvenile foliage on lateral branches, whereas trees undamaged in 1994 produced adult foliage in the upper canopy and relatively few juvenile shoots on lateral branches. Hence, undamaged trees may have been less attractive to ovipositing females.

The proportion of total egg batches collected from each damage type changed with time, suggesting a tendency for moths to disperse within the plantation. Perhaps adult moths dispersed out of the hotspot area and into surrounding bays, particularly in the damaged area, because trees in the hotspot area were so extensively damaged. Since few larvae would have survived to pupate beneath trees sprayed with insecticide in December 1993, it is likely that oviposition in the undamaged area during December 1994 was by females that dispersed into that area after emerging from pupae elsewhere in the plantation.

5.4.4 Implications for population dynamics

According to Price *et al.* (1990), females of eruptive lepidopteran species usually cannot evaluate host plant quality as a resource for larvae "because oviposition is away from larval feeding sites either in time or space and usually both." This study found that *M. privata* females oviposited on expanding young shoots, where larvae soon hatched and began feeding, which is the exact opposite of the above scenario. Such oviposition behaviour is usually associated with latent species (Table 5.5). However, females laid an average of 67.1 ± 2.6 (SE) eggs per batch and appeared unable to discriminate on the basis of whether or not conspecific eggs were present, meaning that, in extreme cases, the number of eggs laid on some leaves was very high (Fig. 5.10). Such oviposition behaviour is usually associated with eruptive

Table 5.5 Characteristics typical of latent and eruptive species (from Price *et al.*, 1990). The final column indicates which of the two categories best describes *M. privata*.

Latent Species	Eruptive Species	*M. privata*
Phenology of adult flight is well synchronised with host plant phenology	Phenology of adult flight is poorly synchronised with host plant phenology	Latent
Females assess resource quality	Females cannot assess resource quality	Latent
Selective oviposition on high-quality young shoots where larvae must establish and feed	Indiscriminate oviposition on old foliage, bark and twigs away from where larvae must establish and feed	Latent
Eggs laid singly or in small numbers	Eggs laid in large numbers	Eruptive
Eggs produced slowly over a female's life	Eggs ready to lay when females emerge	Eruptive
Avoid conspecific eggs	Ignore conspecific eggs	Eruptive
Females good flyers and emigrate from high density sites	Females poor flyers or sedentary	Eruptive
Resources not overexploited	Resources overexploited	Eruptive
Host plants not killed	Host plants killed	Eruptive
No population collapse due to starvation	Population may collapse due to starvation	Eruptive

Figure 5.10 High density of *M. privata* eggs on an *E. nitens* leaf.

species (Table 5.5). Thus, the oviposition behaviour of *M. privata* females has aspects of both latent and eruptive species. Aside from an ability to evaluate host plant quality, suggested by selective oviposition on expanding young shoots of host trees, most other characteristics of *M. privata* at *Wages Rd* were typical of an eruptive species, the other exception being that adult emergence was synchronised with host plant phenology (Table 5.5).

The fact that *M. privata* oviposits directly on expanding young shoots where larvae hatch and begin feeding, yet otherwise typifies an eruptive species, indicates that the central theory of Price *et al.* (1990) is inappropriate for *M. privata*. Therefore, factors other than an inability to evaluate host plant quality must be responsible for *M. privata*'s eruptive population dynamics. Perhaps outbreaks occur only after *M. privata* escapes from its natural enemies (see Appendix A) or after particular climatic requirements (e.g. adequate aitumn rainfall) are met. Once an outbreak has begun, its severity may be intensified by the apparently sedentary nature of females (given their under-representation in sweepnetting results) and their tendency to ignore conspecific eggs during oviposition. Assuming that females can select oviposition sites on the basis of foliage quality, ignoring conspecific eggs

already present at selected oviposition sites may lead to over-exploitation of host plants when population densities of *M. privata* are high. Although it could be argued that intense competition for oviposition sites may have forced females to oviposit on leaves with conspecific eggs, this is considered unlikely. Even when substantial foliage was available for oviposition, multiple egg batches were found on some leaves, while no egg batches were found on others nearby (pers. obs.). Similarly, Tammaru *et al.*, (1995) found that oviposition behaviour of *E. autumnata* was similar in outbreak and latent populations and concluded that oviposition behaviour of that species was uninfluenced by population density.

The fact that some eruptive species exhibit latent species characteristics was acknowledged and discussed by Price *et al.* (1990). They noted that many forest pests with characteristics of latent species have only become pests relatively recently in response to anthropogenic changes in the amount of disturbed forest habitat. They argue that eruptive species that oviposit on vigorous young growth are adapted to exploit disturbed habitats and would remain latent under natural forest conditions where disturbance is infrequent and patchy. However, when human activity creates vast areas of disturbed habitat with vigorous young trees, such as young, even-aged plantations, population eruptions become possible due to increased resource availability for such species. Since *M. privata* oviposits on glaucous juvenile foliage of eucalypts, which under natural conditions would usually be found after site disturbance by fire, this explanation is plausible for *M. privata*. However, although population eruptions are possible when resources are abundant, other factors may also be necessary, such as favourable weather and an absence of natural enemies (Pedigo, 1989). The latter point is illustrated by the fact that damage by *M. privata* in *E. nitens* plantations in Tasmania is sporadic even though the annual planting rate of 5000 ha maintains high resource availability.

5.4.5 Impact of M. privata *defoliation*

Although one-third of the *Wages Rd* plantation was extensively damaged, even more of the plantation may have been defoliated were it not for a water-race running through the plantation (see Fig. 5.1). It is assumed that larvae dispersing in a westerly direction away from the damaged area in their search for new host trees crawled into the water-race and drowned and were thus prevented from spreading any further into the western end of the plantation (G. Holz, North Eucalypt Technologies, pers. comm.). The plantation's western end was only lightly defoliated in the first year of the outbreak (1993-94 summer), presumably by larvae hatching from eggs laid in that area. Marked differences in tree defoliation on opposite sides of the water-race indicate that inter-tree dispersal by larvae contributed to the level of tree damage during the outbreak.

Tree mortality in the hotspot bays, particularly along both sides of the windrow (Fig. 5.9), may have been caused by more severe defoliation there than anywhere else in the plantation. The low hill and large windrow may have benefited larvae by providing physical shelter against westerly winds and adverse weather, possibly resulting in better larval survival and hence, higher defoliation levels. Alternatively, these same factors (sheltered conditions) may have caused adults to congregate or remain in the area, thus leading to higher oviposition levels on trees, higher larval densities and more feeding damage. The nature of this possible 'shelter effect' was not investigated, but appears to warrant further study.

It is generally thought that eucalypt growth during spring and summer depletes starch reserves and that trees heavily defoliated during autumn, when starch reserves are low, are less able to recover (Bamber & Humphreys, 1965; Cremer, 1973; Carne *et al.*, 1974; Abbott & Wills, 1996). Hence, the impact of defoliation on the growth and survival of eucalypts depends largely on the season of defoliation,

with defoliation during late-summer and autumn being the most harmful. Abbott and Wills (1996) found that 50% defoliation of *E. globulus* in autumn significantly reduced height growth and basal area, but the same level in spring and summer did not. Significant long-term effects of 50% defoliation were only found in trees defoliated in autumn. However, complete defoliation of *E. globulus* was harmful at any time, and always resulted in long-term growth reductions. Therefore, since the trees in the hotspot area at *Wages Rd* were severely defoliated by *M. privata* during autumn, it was likely to be particularly harmful.

Mortality of *E. nitens* at *Wages Rd* was higher than the 8% reported by Abbott and Wills (1996) in *E. globulus* defoliated during summer and autumn. In Abbott and Wills' (1996) study, complete defoliation in any season always reduced growth but did not significantly increase plant mortality over controls. In contrast, Cremer (1973) found that complete defoliation of *E. regnans* resulted in significant mortality if trees were defoliated in late summer and autumn. Cremer (1973) noted that tree mortality was not related to the ability to produce new shoots, as many defoliated trees in that study sprouted new shoots, but still died. Thus, the impact of defoliation on mortality of eucalypts may depend on the defoliation level, the host species and the study location. Perhaps severe insect defoliation during late summer and autumn at high altitudes in Tasmania (such as *Wages Rd*) predisposes trees to damage from secondary factors such as frost. A survey of the study site in April 1994 by associates (V. Patel and S. Paterson, unpubl. data) found new buds and shoots sprouting from the branches of defoliated trees, but many of those buds and shoots died during the ensuing winter. Two defoliations in quick succession, the first by *M. privata*, the second by frost, may have contributed to tree mortality in the hotspot area. Thus, it may be premature to attribute tree mortality in the hotspot area entirely to a single, albeit severe, defoliation by *M. privata*.

This chapter has met the four objectives stated at the start of the chapter: (i) the sweepnetting results in December 1994 provide further evidence of early adult phenology at high altitudes in Tasmania; (ii) the study of oviposition locations on host trees revealed that egg batches are more likely to be found on the outermost leaf pairs of expanding young shoots on the sheltered side of the canopy; (iii) the oviposition behaviour of *M. privata* was found to be somewhat intermediate between eruptive and latent species; while (iv) the potential impact of summer outbreaks in high-altitude plantations was dramatically illustrated by the 32% tree mortality in the hotspot bay. By focusing on adult phenology, oviposition behaviour and the impact of severe defoliation by *M. privata* the chapter has virtually ignored the larval feeding period that occurs in between the above 'end stages'. Nevertheless, the chapter has identified behavioural traits which may contribute to the species' eruptive population dynamics and one factor in particular which may contribute to the earlier phenology of *M. privata* at high altitudes in Tasmania, which this PhD is ultimately required to explain. The behavioural traits which may contribute to eruptive population dynamics of *M. privata* are a poor flying ability of females, as they were under-represented in sweepnetting results, and a tendency to oviposit regardless of the presence of conspecific eggs. Finally, low temperatures may be a particularly important abiotic constraint opposing adult emergence later in the season at high altitudes in Tasmania. Early adult phenology at high altitudes in Tasmania may be an adaptation to avoid this abiotic constraint.

6. SUMMER PHENOLOGY ON MAINLAND AUSTRALIA

6.1 INTRODUCTION

One matter yet to be addressed is the summer phenology of *M. privata* in warmer, low altitude areas of its range, such as Victoria. Specifically, the issue of concern is whether *M. privata* poses a risk to young eucalypt plantations during summer in these districts. Such a possibility stems from an early reflective report by Froggatt (1923) in which he claimed that thousands of bushes (presumably young eucalypts) were found to be infested by *M. privata* during summer in the Bendigo region of central Victoria (Fig. 6.1).

Although *M. privata* is summer-active at altitudes above 500 m ASL in Tasmania (de Little, 1981 and see the preceding chapter of this thesis), the above situation was considered unlikely. More recent reports of *M. privata* from Victoria and other mainland states have noted that larvae are usually found during the cooler months of autumn and winter (Roberts & Sawtell 1981; Neumann, 1993; Abbott, 1993; Farrow *et al.*, 1994; Farrow, 1996). Farrow (1996) in particular noted that adults and eggs may occasionally be found during mid-summer in southeastern mainland Australia, but that larvae rarely survive. However, apart from Farrow's (1996) record, it was not clear from the literature how many surveys had specifically looked for *M. privata* in the summer on mainland Australia. Insect surveys, particularly for pest species, tend to concentrate on the period when they are known to be most abundant, so neglecting other periods. Thus, lack of reports of *M. privata* in the summer may have reflected a real absence, but it may also have reflected a poor search effort. For this reason, and also because 25,000 ha of blue gum plantations are currently being planted each year across southern Australia (see Table 1.2), it was considered necessary to survey for *M. privata* in the summer.

Consequently, young blue gum plantations in Victoria were surveyed in early March in two consecutive years (1996-97) for the presence of *M. privata* larvae. The bushland around Bendigo was also surveyed for *M. privata* larvae in March 1997. This chapter presents the findings of those surveys.

6.2 METHODS

6.2.1 Survey dates and localities

Victorian blue gum plantations carrying glaucous juvenile foliage (particularly susceptible to *M. privata* attack) were surveyed for the presence of *M. privata* larvae during the periods 4-8 March 1996 and 3-7 March 1997. Three Victorian districts were surveyed in both years, namely:

1. The South Gippsland region (160 km SE of Melbourne) where most public and private plantations in Victoria are located (Neumann, 1993). The Gippsland plantations were established by Australian Paper Plantations Pty. Ltd. and consisted mainly of *E. globulus*, but included small areas of *E. nitens*.

2. The Shepparton region in northern Victoria where trial plantings of *E. globulus* were established at Shepparton, Nathalia and Cobram by the Victorian Department of Conservation and Natural Resources in 1990

3. Altona in southern Victoria where trial plantings of *E. globulus* were established by CSIRO Division of Forestry in collaboration with Land Energy Pty. Ltd. and Dow Chemical Pty. Ltd. in November 1990 (Farrow *et al.*, 1994).

In addition to the above localities, young eucalypts growing naturally in the bushland around the city of Bendigo were surveyed on 7th March 1997, the objective being to retrace Froggatt's steps during his childhood. The surveyed localities are shown in Fig. 6.1. Climatic details for each locality are given in Table 6.1.

Table 6.1 Victorian localities surveyed for *M. privata* larvae during March 1996 and March 1997. Places in parentheses in the first column indicate the use of meteorological records from stations close to survey localities which had no meteorological records. Climatic details are given for *Surrey Hills* and Hobart in Tasmania for comparative purposes. (Source: Australian Bureau of Meteorology, 1998).

					Mean Daily Air Temperature (°C)			
			Elevation	Mean Annual	January		July	
Locality	Latitude	Longitude	(m ASL)	Rainfall (mm)	Min.	Max.	Min.	Max.
Victoria								
[a] Cobram (Numurkah)	35° 55' S	145° 39' E	110	450	13.6	30.6	2.5	12.9
[a] Nathalia	36° 03' S	145° 12' E	"	"	"	"	"	"
[a] Shepparton	36° 23' S	145° 24' E	"	"	"	"	"	"
Bendigo	36° 46' S	144° 17' E	225	553	14.3	28.9	3.5	12.1
Altona (Laverton)	37° 50'S	144° 48'E	18	566	13.5	25.5	4.9	13.5
Gippsland (Yallourn)	38° 12'S	146° 24'E	155	896	12.1	25.4	3.8	12.8
Tasmania								
Surrey Hills (Waratah)	41° 27' S	145° 32'E	612	2204	6.3	17.6	0.8	7.2
Hobart	42° 53'S	147° 20'E	50	624	11.8	21.5	4.5	11.6

[a] Meteorological records for Numurkah are used for Cobram, Nathalia and Shepparton because Numurkah is approximately 20 km from the three surveyed sites.

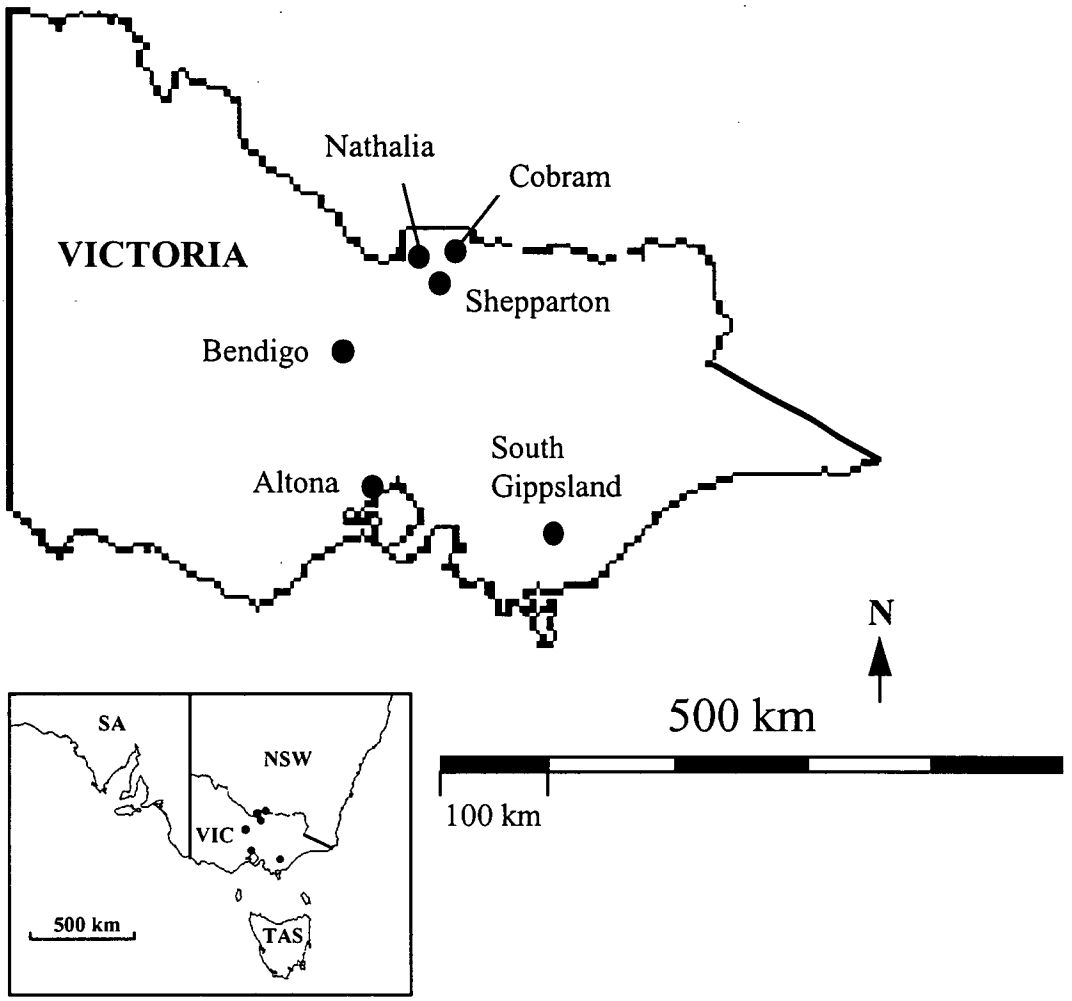


Figure 6.1 Victorian localities surveyed for *M. privata* larvae in March 1996 and March 97. The insert in the bottom left of the figure shows the geographic relationship between Victoria and Tasmania.

6.2.2 Survey Method

A constant period of ten minutes was spent searching for *M. privata* larvae in blue gum plantations (1996 n = 37, 1997 n = 31) during the March survey periods. Assuming a survey rate of one tree every 10-15 seconds, approximately 50 trees were surveyed at each site. Since a higher proportion of eggs was found on the eastern side of the tree canopy in an earlier field study (Section 5.3.2), current-season shoots on the eastern side of tree canopies were surveyed. At each site, the presence or absence of each of the five larval stages of *M. privata* was recorded. Mature caterpillars (L5) on current-season shoots of juvenile blue gums were particularly

sought after as confirmation for Froggatt's (1923) claim that larval infestation could occur during summer. However, the presence of either (*a*) leaf skeletonising around hatched egg batches on current-season shoots or (*b*) parasitoid cocoons next to dead larvae in terminal leaf shelters on current-season shoots were also deemed to be evidence of *M. privata* larval activity during summer. Live specimens of *M. privata* were collected when possible. Fifth instars collected in Victoria were reared to pupation and subsequent adult emergence at 15°C (16L:8D). Fifth instars collected in March 1996 formed larval group 3 in Table 2.3 and gave rise to the Victorian individuals in Figs. 2.8 and 2.9. The occurrence of adults and unhatched eggs was also recorded, but they represented the beginning of autumn activity by the species and were therefore relatively unimportant in the context of this chapter.

6.3 SURVEY RESULTS BY DISTRICT

The presence of *M. privata* adults, eggs and larvae at the surveyed localities of Victoria are presented in Table 6.2. The table shows that adults, eggs, larvae and parasitised larvae were found in Victoria in both years, but not in all areas. The incidence of summer activity in each district is described below:

South Gippsland Region

In March 1996, *M. privata* was found in six of 33 plantations surveyed in the Gippsland region. Adults (n = 2), unhatched eggs and live L1 and L5 were collected at one of the six sites while live L5 were collected at another three of the six sites. Terminal leaf shelters with cocoons of the parasitoid *Apanteles* sp. (Hymenoptera: Braconidae) (Fig. 6.2) next to dead *M. privata* larvae were found at three of the six sites (Table 6.2).

Table 6.2 The results of surveys for *M. privata* adults, eggs and larvae in early March 1996 and 1997. Adult moths and unhatched (U) eggs represent the start of autumn activity. Hatched (H) eggs and larvae represent summer activity.

	March 1996											March 1997										
Locality	No. Sites Surveyed	No. Sites with AGM	Par. Larv.	L5	L4	L3	L2	L1	Eggs [a] H	Eggs [a] U	Adults	No. Sites Surveyed	No. Sites with AGM	Par. Larv.	L5	L4	L3	L2	L1	Eggs [a] H	Eggs [a] U	Adults
Victoria																						
Gippsland	33	6	3	4	0	0	0	1	0	1	1	27	11	3	0	3	1	1	0	7	0	3
Shepparton	1	1	1	1	1	1	1	1	0	1	1	1	1	0	0	1	1	0	0	1	1	1
Nathalia	1	1	0	1	1	1	1	1	0	1	1	1	1	0	0	1	1	1	1	0	1	1
Cobram	1	1	0	1	1	0	0	1	0	0	0	1	0	-	-	-	-	-	-	-	-	-
Altona	1	1	0	0	0	0	0	1	0	1	1	1	1	0	0	0	0	1	0	0	1	0
Bendigo	ns [b]	-	-	-	-	-	-	-	-	-	-	4	0	-	-	-	-	-	-	-	-	-

[a] Hatched eggs were usually found next to small amounts of skeletonising on current-season shoots.
[b] Bendigo was not surveyed (ns) in 1996 because the survey period was cut short by illness.

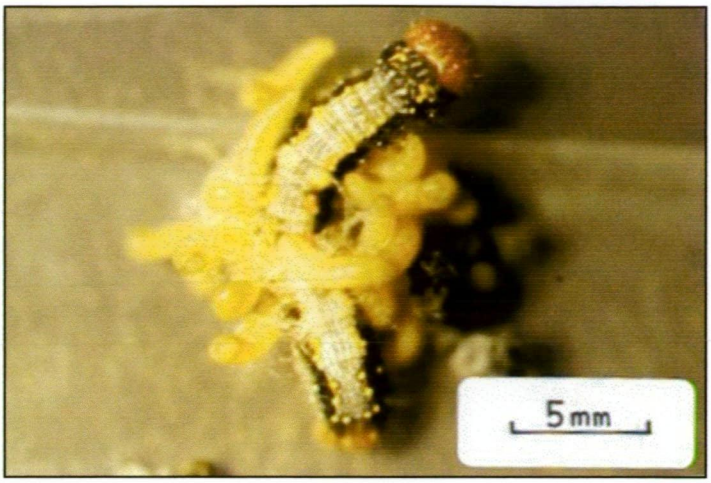


Figure 6.2 Larvae of a braconid parasitoid (*Apanteles* sp.) emerging from a fifth instar *M. privata* larva.

In March 1997, *M. privata* was found in 11 of 27 plantations surveyed. Live caterpillars (L2-L4) were collected from four sites; leaf skeletonising around hatched eggs was found at seven sites; parasitoid cocoons next to dead larvae were found at three sites; while only one or two adults were either collected or observed at three sites. In all instances, *M. privata* activity in the South Gippsland region was found either on a single tree or at most on several of the 50 or so trees surveyed in any ten minute searching period. Based on these records, the incidence of summer activity in the South Gippsland region was considered to be negligible in both years, particularly when compared with levels of summer larval activity observed by the author at high altitude sites in Tasmania between December 1994 and March 1996.

Shepparton, Cobram and Nathalia

In March 1996, adults, eggs and L1-L5 were found in low numbers at both Shepparton and Nathalia (Table 6.2). At Shepparton, one adult in particular was observed on a partially defoliated shoot that had mature L5 in a terminal leaf shelter (Figs. 6.3 and 6.4). This observation indicated that some adults must have emerged and oviposited in mid-summer, which would permit larvae to develop to the 5th

instar by the time the surveys were done. Similarly, parasitoid cocoons found at Shepparton next to dead L4-L5 in leaf shelters further indicated larval development earlier that summer. At Cobram all *E. globulus* trees had predominantly adult foliage with few juvenile shoots remaining in their lower canopies. A single batch of L1 was found skeletonising a juvenile shoot on one tree, while one L4 and several L5 were scattered over a few juvenile shoots on a second tree.

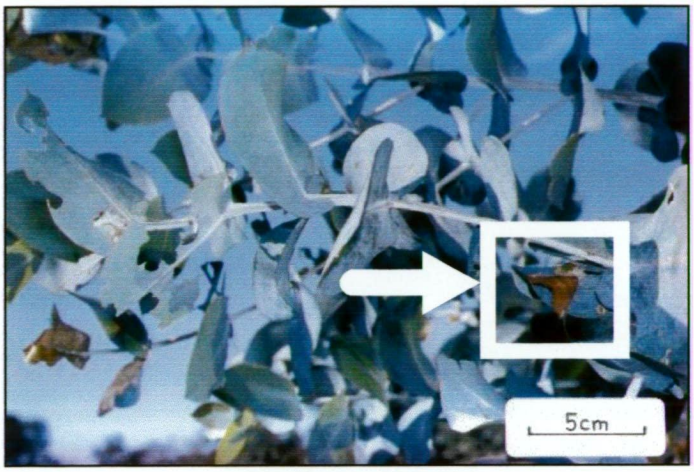


Figure 6.3 Adult *M. privata* (right) on an *E. globulus* shoot partially defoliated by larvae during summer at Shepparton in Victoria. Some adults must have emerged and oviposited in mid-summer in order for larvae to cause the above shoot damage by 7 March 1996 when the photo was taken.

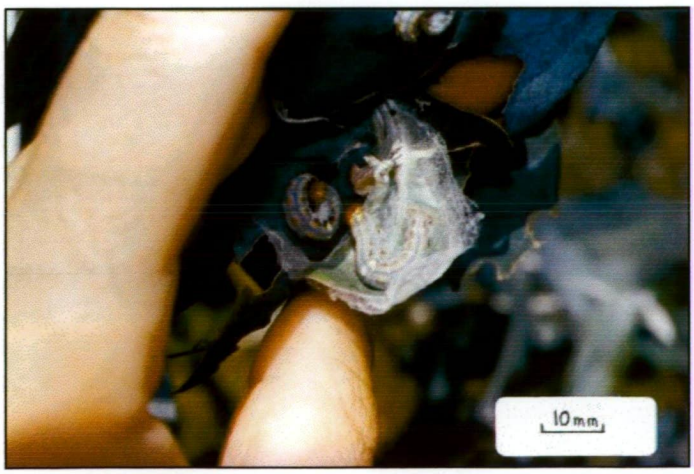


Figure 6.4 Terminal leaf shelter at Shepparton opened up to expose 5th stage *M. privata* larvae. Such larvae were collected and reared to pupation and subsequent adult emergence in the laboratory. Photo taken 7 March, 1996.

In March 1997, adults, hatched and unhatched eggs, L3 and L4 were collected at the Shepparton site while adults, unhatched eggs and L1-L4 were collected at Nathalia. No *M. privata* activity was found at the Cobram site. No 5th instars or parasitoid cocoons next to dead larvae were found at Shepparton, Nathalia or Cobram in March 1997.

Altona

In March 1996, parasitised eggs, developing eggs and L1 were collected from residual juvenile shoots of *E. globulus* with predominantly adult foliage. Two adults and a fresh egg batch were collected from *E. grandis* trees planted alongside the block of *E. globulus*. In March 1997, several batches of eggs and L2 were collected from *E. globulus* and *E. grandis*. No L3-L5 were found at Altona in either year.

Bendigo

Four bushland sites around Bendigo were surveyed on 7th March 1997. At each site, no evidence of *M. privata* was found, but large numbers of another lepidopteran defoliator, the black slug cup moth *Doratifera casta* Scott (Lepidoptera: Limacodidae) (Fig. 6.5), were found feeding on many saplings of one eucalypt species (not identified) prominent in the area. *Doratifera casta* larvae were particularly abundant in comparison to levels of *M. privata* larval activity found elsewhere in Victoria during the March surveys. Terminal shoots from the eucalypt host species of *D. casta* and also from another eucalypt species found in the region were given to *M. privata* larvae collected earlier from Nathalia. Both species were found to be unacceptable for *M. privata* larval feeding (Fig. 6.6).

Figure 6.5 Groups of black slug cup moth larvae (*Doratifera casta*) were found feeding on many saplings of one eucalypt species which was prominent in the Bendigo region.

Figure 6.6 The acceptability of foliage presented to *M. privata* larvae. Two shoots from eucalypts growing naturally in the Bendigo region (left and centre) were unsuitable for larval feeding. The heavily skeletonised shoot on the right is an *E. nitens* shoot given to larvae at the same time. Scale = 10 cm.

6.3.1 High temperature mortality

In the second year that surveys were conducted, small levels of skeletonising around hatched egg batches were occasionally found on current-season shoots which had no other signs of larval feeding activity. Those observations indicated that eggs could develop and hatch during summer, but that larval survival was low. On the basis of those observations, it was hypothesised that poor larval survival was due to the adverse affects of high summer temperatures. To test this hypothesis, the development and survival of eggs and larvae were assessed in the laboratory at a fluctuating temperature of 30/15°C (8L:16D), equivalent to a mean daily temperature of 20°C. The fluctuating temperature regime was designed to simulate mean daily maximum and minimum temperatures at Bendigo and in the Shepparton region during mid-summer (see Table 6.1). Eggs and larvae were also reared at 15°C as controls against which to assess performance at the fluctuating temperature. The

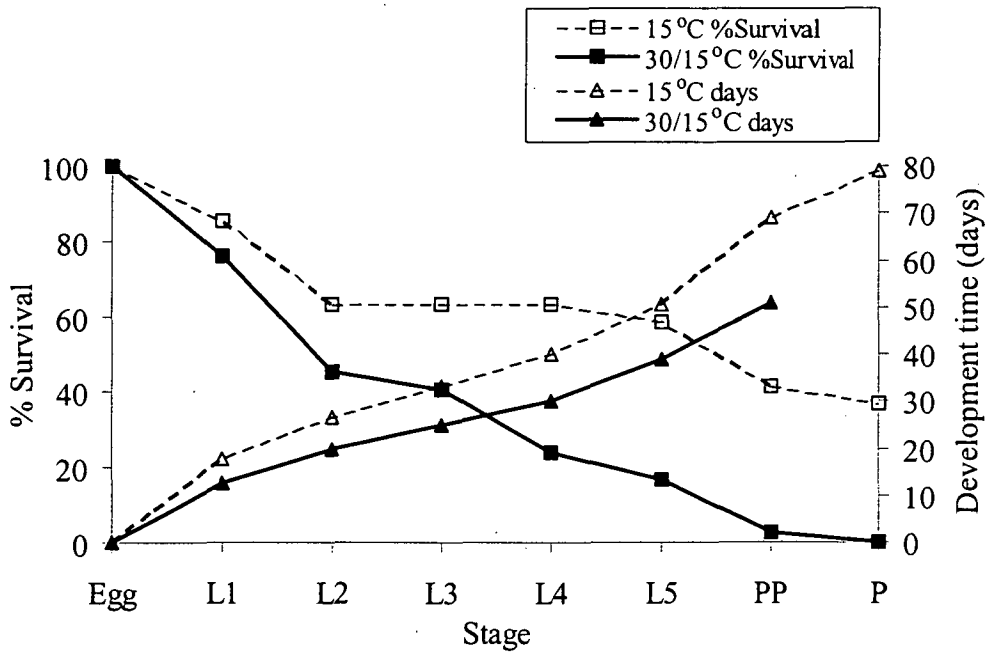


Figure 6.7 The developmental time and cumulative percentage survival of *M. privata* from oviposition to pupation at a fluctuating temperature of 30/15°C (8L:16D) (equivalent to a mean daily temperature of 20°C) and at 15°C (16L:8D) (controls). Solid lines refer to insects at 30/15°C, broken lines to insects at 15°C.

results are presented in Fig. 6.7. The developmental time at 30/15°C was about 25% faster than at 15°C but the percentage survival was lower. No larvae survived to pupation at 30/15°C compared with about 40% survival to pupation at 15°C.

6.4 DISCUSSION

Evidence of summer larval activity by *M. privata* was found in the South Gippsland and Shepparton districts in early March in 1996 and 1997. However, the observed levels in those two districts were considered negligible compared with levels of summer larval activity observed in high-altitude *E. nitens* plantations at *Surrey Hills* and Tarraleah in Tasmania between December 1994 and March 1996. Autumn gum moth was also found at Altona in both years but the stages found (adults, unhatched eggs and L1-L2) probably represented the beginning of an autumn population rather than the end of a summer population. No evidence of *M. privata* was found at Bendigo in March 1997. In general, the survey results provide only limited support for Froggatt's (1923) claim but are consistent with observations by Farrow (1996) that although adult *M. privata* occasionally emerge and oviposit in mid-summer in southeastern mainland Australia, larvae rarely survive. The absence of *M. privata* at Bendigo in particular provided no support for Froggatt's claim.

The most interesting finding from the surveys was the abundance of black slug cup moth larvae feeding on eucalypt saplings growing naturally in the bushland around Bendigo. The abundance of *D. casta* larvae, together with the complete absence of *M. privata* larvae, the unsuitability of leaves from local eucalypts for *M. privata* larval feeding (Fig. 6.6) and consideration of the summer climate at Bendigo (see below) suggest that Froggatt (1923) may have been mistaken. Perhaps the larval infestations seen in the Bendigo region in summer by Froggatt during his childhood were actually *D. casta* larvae and not *M. privata* larvae as remembered.

Both are gregarious feeders that skeletonise the leaf surface as young larvae and become edge feeders as they mature. Froggatt was born in 1858 and would have been at least 60 years old when writing his 'Forest Insects of Australia'. Thus, his 'boyhood' recollections would have been ≈ 45 years old and he may have confused the damage of the less common cup moth with the more common gum moth.

The survey results are consistent with what would be expected on the basis of laboratory results presented in Chapters 3 and 4 of this thesis. Specifically, sections 3.4.5 and 4.4.4 identified pupal aestivation at high temperatures (>18°C) as an important mechanism which permits *M. privata* to delay adult emergence at high temperatures. In the field, pupal aestivation would provide a means to delay adult emergence until autumn in warm regions (see in particular Figs. 4.9 and 4.11). For this reason, it is considered highly unlikely that large summer populations of *M. privata* would be found on mainland Australia.

A climatic comparison between Bendigo and summer- and autumn-emergence sites of *M. privata* in Tasmania also suggests that summer larval activity at Bendigo is unlikely. In Tasmania, summer populations of *M. privata* occur at altitudes above 500 m ASL (e.g. *Surrey Hills*) where mean daily temperatures are mild (10-12°C) in summer and below the species' developmental threshold of 5°C in winter (Fig. 6.8). In contrast, autumn-winter populations occur in lowland areas (e.g. Hobart) where mean daily temperatures are relatively high (15-17°C) in summer (c.f. summer-emergence sites) and above the species' developmental threshold in winter (Fig. 6.8). Based on the assumption that *M. privata* is winter-active at Hobart because summer temperatures are too high, it is considered unlikely that *M. privata* would be summer-active at Bendigo since mean daily temperatures in summer there are even higher (Fig. 6.8). Moreover, since winter temperatures in Bendigo and

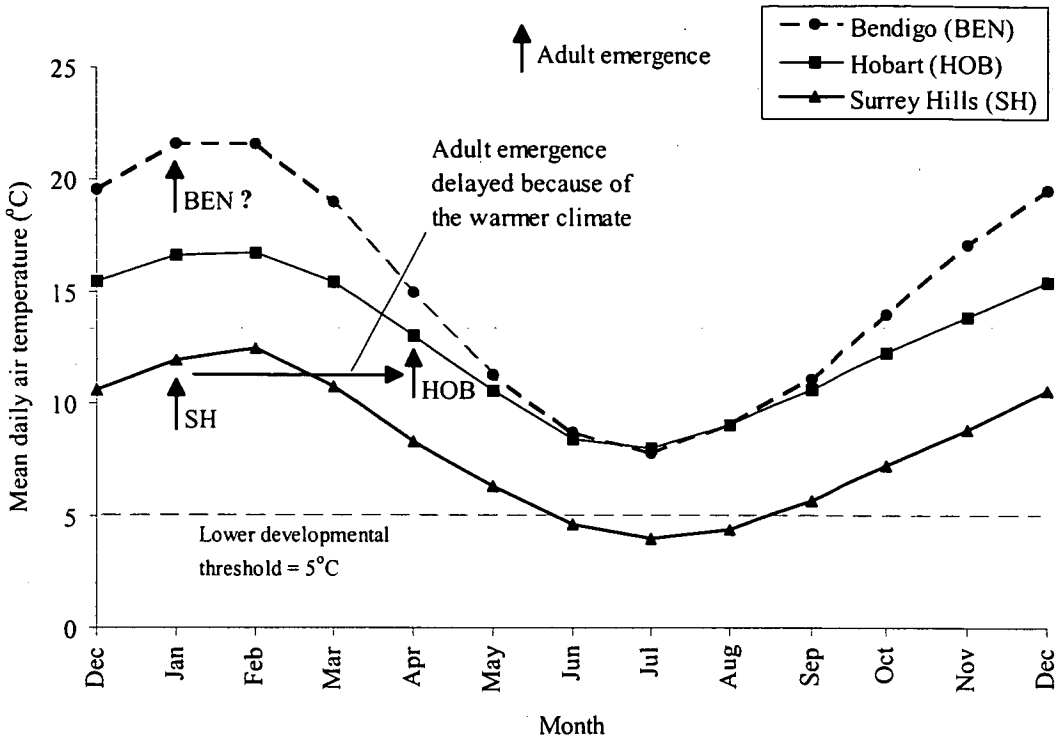


Figure 6.8 Mean daily temperatures for each month of the year for Bendigo in Victoria and for *Surrey Hills* and Hobart in Tasmania. Adults emerge in summer at *Surrey Hills* because of the cool climate but delay emergence until autumn at Hobart because of the warm climate. Given that Bendigo is even warmer than Hobart, it is considered unlikely that *M. privata* would be summer-active at Bendigo.

Hobart are very similar, it seems reasonable to suppose that if *M. privata* were present at Bendigo, it would be winter active.

Although large summer populations of *M. privata* are considered unlikely on mainland Australia, low-level summer populations *are* considered likely. Laboratory results presented in Section 3.4.5 found that adults occasionally emerged rapidly at high temperatures because of variability in the aestivation period. Early termination of aestivation or failure to aestivate after pupal diapause ends would cause adults to emerge in summer in warm regions. Thus, adults emerging at the 'wrong' time for their area (i.e. summer in warm regions) could give rise to low-level summer populations and explain why adults and eggs are occasionally found in mid-summer on mainland Australia (Farrow, 1996).

Whilst the occasional adult may emerge and oviposit in summer on mainland Australia, survey results and the results of a high-temperature larval-rearing experiment indicate that larval survival in summer is likely to be very low. When larvae were reared at a fluctuating temperature of 30/15°C (8L:16D), development time was about 25% faster than at 15°C, but no larvae survived to pupation (c.f. 40% survival to pupation at 15°C) (Fig. 6.7). Thus, regular exposure to maximum temperatures ≥ 30°C during summer could contribute to poor larval survival in summer on mainland Australia.

In addition to climatic constraints such as high temperatures, biotic constraints such as larval parasitism and predation by natural enemies would also contribute to poor larval survival. Cocoons of the braconid parasitoid *Apanteles* sp. were occasionally found next to dead 4th and 5th instars inside leaf shelters on current-season shoots, indicating that some *M. privata* larvae that survived the summer heat were ultimately killed by parasitoids before pupation. Several 4th and 5th instars collected during the surveys were also killed by *Apanteles* sp. before pupation (Fig. 6.2). In addition to this toll, several individuals were destroyed after pupation by the ichneumonid larval-pupal parasitoids *Heteropelma scaposum* and *Anacis* sp. (see Appendix A). The author has also observed predation of *M. privata* larvae by spiders during late summer and autumn at Hobart. The species preying upon *M. privata* larvae closely resembled a member of the genus *Diaea* in the flower spider family Thomisidae (Plate 4 on p. 109 of Mascord's (1980) 'Spiders of Australia'). Subsequent tests in the laboratory confirmed that this spider preyed upon *M. privata* larvae. The combination of climatic and biotic constraints faced by *M. privata* in summer probably prevents large larval populations from building up in summer on mainland Australia.

In conclusion, the surveys found a very low incidence of summer larval activity by *M. privata* in Victoria but the level of activity was negligible when compared with levels found earlier in this study at *Surrey Hills* in the 1994-95 summer (reported in Chapter 5) and at Tarraleah in the 1995-96 summer (where many mature larvae were collected in order to study adult phenology) (Chapter 2). Despite the fact that surveys were only carried out in two years, it is considered highly unlikely that *M. privata* could cause serious damage to young blue gum plantations on mainland Australia during summer. These findings would strongly suggest that Froggatt (1923) may have mistaken *D. casta* larvae feeding on young eucalypts in Bendigo for *M. privata* larvae.

7. GENERAL DISCUSSION

7.1 ORIGINAL OBJECTIVES

Prior to this study, the most detailed pieces of work in relation to *M. privata* phenology were the two life-history studies of *M. privata* done in Tasmania by Elliott and Bashford (1978) and de Little (1981). Both studies have been referred to repeatedly throughout this thesis because they represent the opposite ends of the spectrum of the issue that this PhD project was designed to investigate – namely, geographic variation in the phenology of *M. privata*. Elliott and Bashford's (1978) study documented the autumn-winter life-cycle of *M. privata* in southern Tasmania while de Little's (1981) study reported the contrasting summer-autumn life-cycle of *M. privata* in *E. nitens* plantations at higher altitudes in north-western Tasmania.

Since these studies, damage by *M. privata* has been reported in young eucalypt plantations in all southern states of Australia (Abbott, 1993; Bashford, 1993; Neumann, 1993; Phillips, 1993; Farrow *et al.*, 1994; Abbott & Wills, 1996; Neumann & Collett, 1997). These reports reflect a growing awareness of *M. privata* as a defoliating pest of young eucalypt plantations throughout southern Australia as well as a growing need for more information on *M. privata* which can be used to develop an appropriate management strategy for the species.

Although capable of feeding on over 30 species of *Eucalyptus* (Table 1.1), *M. privata* is primarily regarded as a pest of Tasmanian blue gum, *E. globulus*, shining gum, *E. nitens*, and flooded gum, *E. grandis*, which are grown in commercial pulpwood plantations in southern Australia. The glaucous juvenile foliage characteristic of the blue gum and shining gum species is highly susceptible to attack by *M. privata*, as is the adult foliage of flooded gum. In contrast, the non-glaucous adult foliage type of *E. globulus* and *E. nitens* is seldom attacked. Hence, *M. privata*

is a pest of blue gum plantations primarily during their establishment phase, which usually lasts from two to five years (Beadle *et al.*, 1989; Eldridge *et al.*, 1993).

The pest status of *M. privata*, the susceptibility of juvenile blue gum plantations and the contrasting phenology of *M. privata* at high altitudes in Tasmania were highlighted all at once by an outbreak of *M. privata* in a high-altitude, two-year-old *E. nitens* plantation in north-western Tasmania in the 1993-94 summer. This study commenced six months after the extensive damage caused by that outbreak was first detected.

A major difficulty associated with *M. privata* management is detecting large populations before larval feeding causes severe damage to host trees (Farrow *et al.*, 1994; Floyd *et al.*, 1994). Early detection of populations is difficult because outbreaks are sporadic and sometimes localised, and also because the phenology of *M. privata* varies throughout the distribution of the species (see above). For these reasons, the primary objectives of this study were to identify the underlying mechanisms influencing the phenology of *M. privata* and to identify possible causes of geographic variation in the phenology of the species. The unifying hypothesis was that geographic variation in the phenology of *M. privata* is caused by different life-history strategies in response to different climatic environments. It will become evident as this chapter unfolds that the above unifying hypothesis was correct and that sufficient information has now been accumulated to address both key objectives of the study.

7.2 KEY FINDINGS

7.2.1 M. privata *has a flexible life-history strategy influenced by its environment*

When this study commenced, summer breeding by *M. privata* at high altitudes in Tasmania was generally thought by the author to represent seasonal activity which was uncharacteristic and *early* for the species. This perception was based on the assumption that *M. privata* was a winter-active species by nature and that summer activity was therefore 'aberrant'. The species' common name of autumn gum moth contributed to the above perception. Because of its common name, it seemed reasonable to assume that seasonal activity in summer (i.e. before autumn) was early for the species. However, it is now clear from the results of this study that *M. privata* has a highly flexible life-history strategy that can follow several different pathways according to environmental conditions (see in particular Fig. 4.10).

The most common phenological pattern exhibited by *M. privata* is seasonal activity from autumn to spring, followed by summer dormancy. The predominance of this life-cycle probably reflects widespread environmental conditions in southern Australia which are adverse to the survival of *M. privata* larvae in summer (see below) but favourable for their survival in autumn and winter. Not only are eggs and larvae sensitive to temperatures ≥ 30°C (Figs. 3.9 and 6.7 respectively) which are commonly experienced on mainland Australia during summer, larvae in particular are susceptible to natural enemies which presumably are most active at the very same time that *M. privata* larvae would struggle just to survive. Larvae might occasionally survive the challenge of summer heat but their success would often be short-lived, for when mature larvae were collected in early autumn from Victoria (see Chapter 6), they were often found to be parasitised by the braconid *Apanteles* sp. (Fig. 6.2) or by the ichneumonids *Anacis* sp. and *Heteropelma scaposum* (Appendix A). These high costs during summer may prevent *M. privata* from

breeding in summer and make autumn-winter breeding a necessity for continued inhabitation of mainland Australia and lowland areas of Tasmania. Similarly, McQuillan *et al.* (1998) noted that "hot dry summers are a stressful time of year for Lepidoptera because foodplant quality is rapidly declining, eggs and larvae are prone to desiccation and predation by ants is more likely". Thus, climatic and biotic constraints during summer in warm regions may dictate that the start of seasonal activity by *M. privata* be delayed until autumn.

Summer activity on the other hand is uncommon behaviour for the species and is largely restricted to cooler, high-altitude sites in Tasmania. These elevated sites appear to offer sufficient escape from the summer constraints described above to enable *M. privata* to breed in summer. The challenge in elevated areas is to complete larval development before the onset of winter. It is now considered that summer activity by *M. privata* at high altitudes in Tasmania should not be regarded as aberrant behaviour (as previously thought), but as a different life-history strategy caused by local environmental conditions which favour a summer-active life-cycle.

Given that the life-history strategy of *M. privata* is highly flexible, a major challenge faced by individuals is how to synchronise seasonal activity with conspecifics and a favourable environment. As noted by Waldbauer (1978), Dingle (1986) and Danks (1994a, 1994b), insects have many mechanisms available to them to overcome this very challenge. Pupal diapause and pupal aestivation are the primary mechanisms used by *M. privata* to overcome this challenge. The way these mechanisms might be used by *M. privata* to regulate its phenology is presented in the following section.

7.2.2 The ecological roles of diapause and aestivation in M. privata

According to Saunders (1976), winter-active insect species in the northern hemisphere usually enter aestival (summer) diapause in response to long daylengths, whereas summer-active species usually enter hibernal (winter) diapause in response to short daylengths. Throughout its distribution in southern Australia, *M. privata* is predominantly active in autumn and winter and spends summer in the pupal stage. Accordingly, one might reasonably expect that *M. privata*, like winter-active species in the northern hemisphere, would enter an aestival-type diapause in response to long daylengths. However, the results presented in Chapters 2 and 4 clearly indicate that a hibernal-type diapause in *M. privata* pupae is induced by short daylengths and low temperatures experienced during larval development and averted by long daylengths and high temperatures. In addition, the autumnal equinox was found to be an approximate *switching time* separating the alternative developmental pathways (i.e. diapause or non-diapause) taken after pupation (Fig. 2.11). The concept of a switching time was first advanced by Taylor (1986) and provides a fine explanation for the seasonal variation in pupal duration reported in Chapter 2 (Figs. 2.9 and 2.11). As a consequence of the above factors, larvae that developed under longer daylengths in the field before the autumnal equinox or under a 16 hr daylength in the laboratory produced non-diapause pupae, whereas larvae that developed under shorter daylengths in the field after the autumnal equinox or under an 8 hr daylength in the laboratory produced diapause pupae. These results strongly suggest that *M. privata* is a long-day species (Type I of Beck's (1968) classification).

Whilst a hibernal-type diapause in *M. privata* pupae is induced by short daylengths experienced during larval development, the ecological role of pupal diapause varies markedly in different areas of the species' distribution, as described in the two scenarios of Figure 7.1. At high altitude sites in Tasmania, where

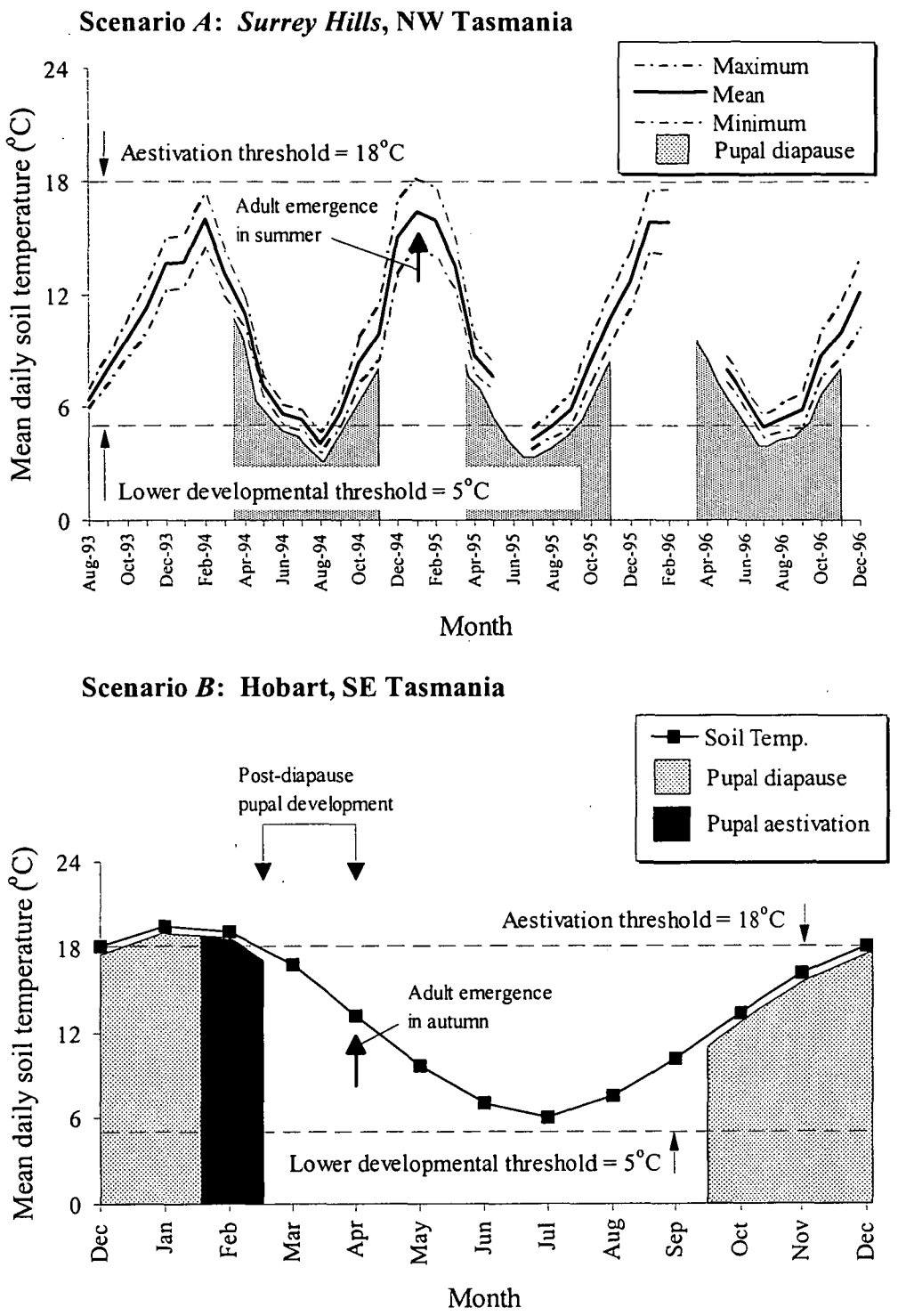


Figure 7.1 Schematic diagram representing how pupal diapause and pupal aestivation might regulate the phenology of *M. privata*. Scenario *A* occurs in cooler, high altitude areas in Tasmania. In this scenario, diapause prevents pupal development before winter and aestivation is unlikely to occur in summer since soil temperatures usually remain below the aestivation threshold. Scenario *B* occurs in warmer regions of southern Australia. In this scenario, diapause prevents pupal development before summer and pupal aestivation is likely to occur if diapause ends before March since soil temperatures usually remain above the aestivation threshold until autumn. Mean soil temperatures are for a depth of 10 cm. Temperatures for *Surrey Hills* kindly supplied by Greg Holz, North Eucalypt Technologies. Temperatures for Hobart supplied by the Hobart Bureau of Meteorology.

populations are active during summer and autumn and overwinter in the pupal stage, diapause regulates the life-cycle in much the same way as described for summer-active species in the northern hemisphere many times previously in the diapause literature (e.g. Danilevskii, 1961, p.17; Tauber *et al.*, 1984, pp. 162-163; Topp, 1994, p. 291). Diapause suppresses development late in the warm season (autumn) when conditions are temporarily favourable for development, but will ultimately be followed by unfavourable conditions during winter. Diapause ends some time during winter or early spring, after which post-diapause development may begin immediately or be further interrupted via a period of quiescence in direct response to continuing low temperatures below the species' lower developmental threshold of approximately 5°C. Once soil temperatures rise above this threshold in spring, post-diapause development begins and adults emerge from pupae once sufficient degree-days are accumulated for adult differentiation (485 DD > 5°C from Table 3.14). Pupal aestivation (Fig. 4.9) probably has no role in the life-cycle at high altitudes in Tasmania because of the cooler climate. This first scenario is illustrated in Fig. 7.1a and for simplicity is hereafter referred to as 'scenario *A*'.

The second scenario (Fig. 7.1b), hereafter referred to as 'scenario *B*', is a reversal of scenario *A* and occurs throughout the remainder of *M. privata*'s distribution, where populations are winter-active and oversummer in the pupal stage. Although pupal diapause is induced by short daylengths as before, instead of suppressing development late in the warm season, diapause suppresses development early in the warm season (i.e. spring) when conditions are temporarily favourable for development, but will ultimately be followed by unfavourable conditions during summer. By initiating diapause immediately after pupation in winter and spring, pupal development is suppressed for several months during spring and summer,

thereby preventing adult emergence and reproduction from occurring in summer when maximum daily temperatures ≥ 30°C may be adverse to the development and survival of eggs and soft-bodied larvae (Figs. 3.9 and 6.7 respectively). Thus, an otherwise redundant hibernal diapause, induced by short daylengths, is used by *M. privata* as a substitute for an aestival diapause (normally induced by long days in truly winter-active species) in warm regions of its distribution. This is a fine example of how the same trait (diapause in response to short daylengths) may be used by a species to solve different ecological problems in different geographic areas, a phenomenon previously noted by Danks (1994b).

Continuing with scenario *B*, pupal diapause in warm regions probably ends some time during summer or early autumn, after which post-diapause pupal development may begin immediately, or as illustrated in Fig. 7.1b, be further interrupted by pupal aestivation (Fig. 4.9) (c.f. low-temperature quiescence in scenario *A*) in direct response to continuing high temperatures above a pupal aestivation threshold of approximately 18°C. Pupal aestivation in *M. privata* is a complicated process because it may occur early, late, early and late or not at all during adult differentiation (Fig. 4.9). The ability to aestivate provides a means to extend pupal dormancy even longer after diapause ends and seems likely to make forecasting of adult phenology particularly difficult in warm regions. However, since aestivation occurs in direct response to high temperatures, it ends rapidly once soil temperatures fall below the aestivation threshold in autumn. Therefore, adult emergence should be anticipated once soil temperatures fall below the aestivation threshold. Theoretically, pupae aestivating as unpigmented pharate adults (Fig. 4.9c) should produce adults earlier than pupae in early-pupal aestivation (Fig. 4.9b). Thus, we might expect *M. privata* to exhibit a bimodal pattern of adult emergence in

autumn in warm regions. Finally, low-temperature quiescence probably seldom occurs during the pupal stage in scenario *B* because the pupal stage occurs during the warmest part of the year (Fig. 7.1b).

The two scenarios above illustrate how geographic variation in the phenology of *M. privata* may be caused by different life-history strategies in response to different climatic environments. Pupal diapause and pupal aestivation are the primary mechanisms regulating the phenology of *M. privata*. Waldbauer (1978) considered diapause a 'coarse' adjustment mechanism that simply prevents further development until the next favourable growing season, but also noted that insects may have 'fine' and even 'ultra-fine' adjustment mechanisms which contribute to synchronised emergence. I agree with Waldbauer (1978) that diapause is a coarse adjustment mechanism. In *M. privata* it prevents the initiation of adult differentiation until the next favourable season, be it spring at high altitudes in Tasmania (Fig. 7.1a), or late summer in warmer regions (Fig. 7.1b). Once diapause has ended, fine adjustment to adult emergence times may be achieved in post-diapause populations via lower and upper temperature thresholds for the resumption of development. These thresholds are the lower developmental threshold and the pupal aestivation threshold, approximately 5°C and 18°C respectively. Local populations of post-diapause pupae may initiate development in synchrony when temperatures first become favourable, either by rising above the lower threshold in spring in cool regions, or falling below the aestivation threshold in autumn in warm regions. Thereafter, ultra-fine adjustment of individual emergence times may occur via microclimatic effects on the developmental rates of individual pupae.

Together, pupal diapause and pupal aestivation provide a means for considerable flexibility in the life-cycle of *M. privata* and enable different geographic

populations to vary their phenology to best match the local environmental conditions. As environmental conditions change over the distribution of the species, diapause and aestivation interact with local conditions and cause temporal shifts in phenology which match the life-cycle to the local environment. Adults emerge in summer at high altitudes in Tasmania because the climate is cool and pupae do not aestivate in summer (Fig. 7.1a). In contrast, adult emergence is delayed until autumn elsewhere in southern Australia because the climate is warm and pupae aestivate in summer if diapause ends too early (Fig. 7.1b).

Denlinger (1985) stated that diapause is usually expressed as a threshold character in most species and that diapause intensity (or duration) seldom varies, but noted that there were some exceptions. In contrast, Danks (1994a) noted that many species entering a diapause later in the season remain in diapause for a shorter period. The findings of this study agree with Danks (1994a). The pupal duration of *M. privata* at 15°C declined from about eight months following larval collection in June to four months following larval collection in November (Fig. 2.8a). Since adult differentiation took approximately two months at 15°C, this result reflected a seasonal decline in the duration of pupal diapause from approximately six months to two months between June and November respectively (Fig. 2.11). The seasonal decline in diapause duration contributed to relatively synchronised adult eclosion the following autumn (Fig. 2.8b).

The most likely cause of the seasonal decline in the duration of pupal diapause in *M. privata* is an inverse relationship between diapause duration and the number of short days accumulated above the required day number (RDN) (see Saunders, 1978 or Takeda & Skopik, 1997). Larvae completing their development early in the season (but after the switching time discussed above) would

accumulate fewer short days above the RDN. The low number of short days experienced would signal that pupation was early and hence, that a longer diapause was required. In contrast, larvae completing their development late in the season would accumulate more short days above the RDN. This would signal that pupation was late and hence, that a shorter diapause was required.

Another conclusion that may be drawn from the discussion thus far is that geographic variation in the phenology of *M. privata* is probably due to phenotypic plasticity and not to genetic differences between populations. Phenotypic differences occur in a range of different characters in many organisms. These include morphological, physiological, biochemical and behavioural differences (Moran, 1992; Via, 1994). Phenotypic variation in these characters may occur because of genetic differences, where different phenotypes are due to different genotypes, or in response to environmental differences, where a single genotype gives rise to different phenotypes depending on the environment experienced (Nylin, 1994; Via, 1994; Nylin & Gotthard, 1998). The latter is known as phenotypic plasticity and is a major cause of phenotypic variation among many organisms (Nylin, 1994). Therefore, if it can be shown that alternative phenotypes are produced primarily in response to different environments, we would have evidence for phenotypic plasticity. From the above discussion, it is clear that alternative behavioural phenotypes in *M. privata* (summer and autumn emergence) are associated with different climatic environments. Thus, we have strong evidence that geographic variation in *M. privata* phenology is due to phenotypic plasticity.

7.3 A COMPARISON BETWEEN *M. privata* AND OTHER INSECT SPECIES

Up until this point, the discussion has focused on the diapause and aestivation behaviour of *M. privata*, the intention being to propose without distraction how

diapause and aestivation interact to influence the phenology of the species. Having proposed the ecological roles of diapause and aestivation in *M. privata* as clearly as possible in the preceding section, it is now possible to make comparisons between *M. privata* and other insect species. However, since insect phenology is a very large field, for simplicity I have chosen to restrict the discussion to the regulation of lepidopteran phenology in particular.

The findings of this study are related first to four 'model' lepidopteran species, namely *Abraxis miranda* (Butler) (Geometridae), *Bombyx mori* L. (Bombycidae), *Agrotis infusa* (Boisd.) (Noctuidae) and *Mamestra brassicae* L. (Noctuidae) which I believe that most readers will be familiar with. Then, the findings are placed into the context of studies which have investigated factors controlling the phenologies of two northern hemisphere geometrids, namely the autumnal moth *Epirrita autumnata* and the winter moth *Operophtera brumata* which share *M. privata*'s unusual habit of emerging and ovipositing during the cooler months of the year.

Since the phenology of another winter-active geometrid, *Abraxis miranda*, was often described in the early diapause literature, (reviewed by Danilevskii, 1961; Beck, 1968 and Saunders, 1976) it is fitting to begin by comparing the phenology of *M. privata* with that of *A. miranda*. *Abraxis miranda* is a bivoltine species that restricts its development to the cooler months of the year (reviewed in Danilevskii, 1961, pp. 206-208). The species exhibits an aestival diapause in the pupal stage and, like true winter-active species (see Saunders, 1976), diapause is induced by long days and high temperatures experienced during larval development. The first generation develops under long days and high temperatures in spring, so pupae enter an aestival diapause that lasts until autumn. The second generation begins when temperatures

fall in autumn and, since larvae of that generation develop under short days and low temperatures in winter, they produce non-diapause pupae that develop immediately after temperatures rise the following spring. The autumn-winter life-cycle of *M. privata* resembles the second generation of *A. miranda*, but in contrast to *A. miranda*, diapause in *M. privata* is induced by short instead of long daylengths. This means that *M. privata* enters a diapause immediately after pupation in winter or spring, whereas *A. miranda* must complete a spring generation before pupae can enter a diapause sufficient to carry that species over the summer months. This comparison illustrates how a hibernal diapause (induced by short days) is used by *M. privata* as a substitute for an aestival diapause that in other winter-active species is usually induced by long days.

The second model species is the commercial silkmoth, *Bombyx mori*, a species renowned for its complex mechanism of diapause induction (reviewed by Danilevskii, 1961, pp. 204 and Saunders, 1976, p.90). *Bombyx mori* is a bivoltine, summer-active species that diapauses in the egg stage. However, despite being a summer-active species, diapause is induced by long days and high temperatures experienced by eggs and young larvae of the maternal generation and averted by short days and low temperatures. Overwintering eggs that give rise to the first generation develop in early spring under short days and low temperatures (see Danilevskii, 1961). Hence, first generation adults lay non-diapause eggs. Second generation eggs develop in summer when days are long and temperatures high. Hence, second generation adults lay diapausing eggs that overwinter. Like *M. privata*, *B. mori* has a photoperiodic response which is opposite to that expected on the basis of its seasonal development pattern. *M. privata* is a predominantly winter-active species with a diapause that is induced by short days, whereas *B. mori* is a summer-active species with a diapause that is induced by long days.

The third model species is the Bogong moth, *Agrotis infusa*, a species renowned for its oversummering behaviour (Common, 1954). *Agrotis infusa* and *M. privata* both reproduce in autumn and exhibit larval development in winter, but the oversummering behaviour of the two species is very different. Sexually immature adult *A. infusa* emerge in spring and migrate from their breeding grounds in southeastern Australia to the Australian Alps, where they aestivate from November to February in rock crevices and small caves (Common, 1954). In contrast, autumn-breeding populations of *M. privata* probably oversummer in a state of pupal diapause in their breeding ground. However, if diapause ends when temperatures are adversely high, pupal aestivation provides a means to extend the pupal stage until autumn. When adult *M. privata* emerge, they are sexually mature and may begin reproducing within 1-2 days of emergence (pers. obs. in the laboratory). Thus, in simple terms, *A. infusa* emerges before aestivating, whereas *M. privata* aestivates before emerging. The end result is the same – both species delay reproduction until autumn.

The fourth and final model species is the cabbage moth, *Mamestra brassicae*, renowned because it displays both hibernal diapause and aestival dormancy in the pupal stage (Sauer *et al.*, 1986; Grüner & Sauer, 1988; Grüner & Masaki, 1994). Hibernal diapause in *M. brassicae* pupae is induced by short days and low temperatures experienced during larval development and terminated by a period of chilling (of the pupa). In contrast, aestival dormancy is a modified form of non-diapause development (Sauer *et al.*, 1986) which is induced by warm, long day conditions during larval development and is terminated rapidly under cool conditions. Like the cabbage moth, autumn gum moth (AGM) displays both hibernal diapause and aestival dormancy in the pupal stage. However, whilst hibernal

diapause in both species is induced by short days and low temperatures experienced during larval development, induction of aestival dormancy appears to differ between the two species. Whereas long days and high temperatures during larval development induce aestival dormancy in cabbage moth pupae, it appears that aestival dormancy in AGM pupae is a direct response by pupae to their immediate thermal environment. Generally, low temperatures (5-18°C) lead to continuous pupal development, high temperatures (> 18°C) to aestivation, providing that diapause has ended. Furthermore, whereas aestival dormancy in AGM pupae can occur both before and during adult differentiation (Fig. 4.9), aestival dormancy in cabbage moth pupae appears to occur only before adult differentiation begins. Grüner and Masaki (1994) stated that at 25°C, *M. brassicae* adults usually emerged about 8-9 days after eye pigmentation became visible through the pupal cuticle. The period when eye pigmentation is visible in *M. brassicae* pupae is probably equivalent to the black-eye phase during the development of *M. privata* pupae (Fig. 2.4b). Hence, cabbage moth pupae have only been reported to aestivate before the black-eye phase, whereas AGM pupae can aestivate both before and after the black-eye phase. Thus, aestival dormancy in cabbage moth pupae appears to resemble the early-pupal aestivation discovered in AGM pupae (Fig. 4.9b).

Perhaps the most striking difference between these species is that in the cabbage moth, hibernal diapause occurs in winter in one generation and aestival dormancy occurs in summer in another generation (see Fig. 6 in Grüner & Masaki, 1994), whereas in AGM, hibernal diapause and aestival dormancy may occur one after the other in pupae of a single generation.

The following discussion compares the mechanisms regulating the phenology of *M. privata* with those regulating the phenologies of the autumnal and winter

moths, *E. autumnata* and *O. brumata* respectively. As noted previously, these northern hemisphere geometrids share *M. privata*'s unusual habit of emerging from pupation and ovipositing during the cooler months of the year. Climate is a major factor maintaining the phenology of both northern hemisphere geometrids (Holliday, 1985; Peterson & Nilssen, 1996). Specifically, adult emergence of both species is delayed until autumn or early winter because summer temperatures are normally above-optimal and therefore inhibit pupal development (Wylie, 1960; Holliday, 1985; Topp & Kirsten, 1991; Peterson & Nilssen, 1996).

This study found that high temperatures in summer are also likely to inhibit the development of *M. privata* pupae in warm regions (see scenario *B* and Fig. 7.1b above). Like the northern hemisphere geometrids, development rates of *M. privata* pupae were optimal at intermediate temperatures and inhibited at temperatures above and below the optimum temperature (Fig. 3.14). Furthermore, the inhibitory effect of high temperatures was largely restricted to the second half of adult differentiation in pupae of both *M. privata* (Fig. 3.18) and *O. brumata* (Wylie, 1960). Wylie (1960) found that 6.5°C was more favourable than 18°C for development late in the pupal stage and concluded that the optimum temperature for rapid development decreased as winter moth pupae matured. A similar phenomenon was found in *M. privata* pupae since stage-2 development rates were often faster at lower temperatures (15-18°C) than at higher temperatures (20-24°C) (Fig. 3.18). Thus, high-temperature inhibition of pupal development appears to be a common mechanism used by all three geometrid species to ensure that adult emergence is delayed until autumn or early winter in warmer regions of their respective distributions.

7.4 PRACTICAL IMPORTANCE OF THE FINDINGS

In this final section of the thesis, I make some recommendations as to how the findings of this study might be integrated into a pest management strategy for *M. privata*. I begin by identify the general outbreak characteristics and management requirements for *M. privata*. Shepherd (1994) reviewed the outbreak characteristics of common forest insect pests in British Columbia and distinguished two general categories. These were forest pests with fast-cycling outbreaks and forest pests with sustained outbreaks. Alternative management strategies were recommended for each category. Since four of the six species given as examples of fast-cycling pest species were geometrids, Shepherd's (1994) description of fast-cycling species may be highly appropriate for *M. privata*:

> "Species with fast-cycling outbreaks rise quickly to visible defoliation levels, cause significant growth loss, tree deformation and mortality, and disappear just as quickly. Impact is closely related to the severity of defoliation during the first year of an outbreak"

The results presented in Chapter 5 of this thesis illustrate the impact that severe defoliation by *M. privata* during the first year of an outbreak had on a two-year-old *E. nitens* plantation in NW Tasmania. Figure 5.9 in particular shows that many trees in the epicentre of the outbreak died after a single defoliation, although as noted at the end of that chapter, frost may have compounded the effect of insect defoliation on host trees. Since *M. privata* is a fast-cycling species, the management strategy recommended by Shepherd (1994) for such species may also be highly appropriate:

> "The objective of managing these species should be to reduce populations before defoliation occurs, i.e. to prevent the outbreak. To accomplish this objective, identification of susceptible habitats and monitoring with sensitive pheromone traps in areas of expected outbreaks are necessary to detect upwelling populations... . [However] if populations rise to outbreak levels undetected, choices are reduced and a fast-acting insecticide may be needed to reduce populations before they cause significant damage."

Thus, we should aim to (a) identify susceptible habitats, (b) detect upwelling populations early, and (c) take action to prevent the outbreak. We already know that habitats susceptible to *M. privata* are young blue gum plantations. We also know that large populations of *M. privata* are easily controlled by the application of fast-acting insecticides (Neumann & Collett, 1997). This leaves early detection of building populations as the major challenge in relation to *M. privata* management.

Shepherd (1994) noted that most of the fast-cycling species overwinter as eggs and hatch in spring to feed on flushed foliage. This pattern is exhibited by the winter moth (Holliday, 1985) and the autumnal moth (Kaitaniemi *et al.*, 1997). This means that the lead time between adult emergence and oviposition in autumn and the onset of damage by those species in spring is usually 3-4 months. Despite a similar adult phenology, the phenology of egg-larval development of *M. privata* is very different from that of the two northern-hemisphere geometrids and other fast-cycling species. Eggs of *M. privata* are usually laid in autumn, but they do not overwinter. Instead, eggs develop directly and hatch in autumn and larvae feed from autumn to early winter, occasionally until early spring. Larval development of *M. privata* is possible during winter in most areas of its distribution because mean daily temperatures in winter are usually above its lower developmental threshold (Fig. 3.22) and its host plants, *Eucalyptus* spp., are evergreen. Thus, in contrast to northern-hemisphere eruptive species, which remain in the egg stage for 3-4 months between oviposition and hatching, there is usually only several weeks lead-time between oviposition by *M. privata* in autumn and the onset of hatching and subsequent defoliation. This means that it is imperative to detect large populations of *M. privata* as soon as possible after oviposition.

The series of temperature-rate studies presented in Chapter 3 determined that in the absence of pupal diapause and pupal aestivation, the generation time of *M. privata* is ca. 1268 degree-days (DD) above a developmental threshold of ca. 5°C, consisting of 784 DD for egg-larval development and 484 DD for pupal development (Table 3.14). However, since over 90% of damage by *M. privata* occurs during the final two instars (L4-L5) of the larval stage (Phillips, 1996), the period between oviposition and the fourth instar, or 'time-to-L4', is especially important as it represents the lead time between peak oviposition and the onset of severe damage. Time-to-L4 required approximately 390 DD > 5°C (Table 3.11). This means that at mean daily temperatures of around 12°C, typical of field temperatures experienced by *M. privata* during egg-larval development (Fig. 3.22), time-to-L4 is predicted to be about 56 days (Table 3.20). Hence, large populations of *M. privata* must be detected and controlled within about two months of peak oviposition in order to minimise damage to host trees. The flow charts presented at the end of Chapters 3 and 4 (Figs. 3.27 and 4.10 respectively) provide models which could be used as a basis for developing monitoring systems aimed at detecting the start of seasonal activity of *M. privata* in susceptible plantations. Furthermore, the development of pheromone traps for *M. privata* which could be used in conjunction with these phenological models would appear to be the next major step towards developing a monitoring system specifically targeting *M. privata*.

It is fitting to conclude this thesis by assessing its contribution to the broader objective of improving management systems for native pests of eucalypt plantations in Australia. In an assessment of the future role of insect pest management in intensively-managed eucalypt plantations in Australia, Ohmart (1990) considered that the limited ecological knowledge of many eucalypt-feeding insects in Australia

was a major problem. To overcome that problem, Ohmart (1990) recommended that more studies on the life histories of many eucalypt-feeding insects be encouraged. This study has made significant inroads into the understanding of the phenology and life-history of the autumn gum moth *M. privata* (see in particular Fig. 4.10) and therefore contributes towards this broader objective advanced by Ohmart (1990). The findings of this study will provide a basis upon which (a) good predictions may be made in relation to *M. privata* phenology and (b) an appropriate management strategy for *M. privata*, based on a solid understanding of the species' phenology, might be developed in the near future.

REFERENCES

ABARE (1998). Australian Forest Products Statistics, September quarter 1997. Commonwealth of Australia, Canberra. 84 pp.

Abbott, I. (1993). Insect pest problems of eucalypt plantations in Australia. 6. Western Australia. *Aust. For.* 56(4): 381-384.

Abbott, I. and Wills, A. (1996). Growth of young *Eucalyptus globulus* in plantations after manual defoliation simulating insect herbivory. *CALMScience* 2: 129-132.

Allsopp, P. G., Daglish, G. J., Taylor, M. F. J. and Gregg, P. C. (1991). Measuring development of *Heliothis* species. In: Zalucki, M. P. (Ed.) *Heliothis*: Research Methods and Prospects. Springer-Verlag, New York. pp. 90-101.

Askew, R. R. (1971). Parasitic Insects. Heinemann Educational Books Ltd., London. 316 pp.

Australian Bureau of Meteorology (1995). Geography. Ch. 3 In: Australian Bureau of Statistics (Ed.) Tasmanian Year Book 1996. Commonwealth of Australia, Hobart. pp. 35-45.

Australian Bureau of Meteorology (1998). World-Wide-Web page on the Internet. http://www.BoM.GOV.AU/climate/averages

Australian Bureau of Statistics (1997). Year Book Australia 1997. Australian Government Publishing Service, Canberra. 767 pp.

Bamber, P. K. and Humphreys, F. R. (1965). Variations in sapwood starch levels in some Australian Forest species. *Aust. For.* 29: 15-23.

Bashford, R. (1993). Insect pest problems of eucalypt plantations in Australia. 4. Tasmania. *Aust. For.* 56(4): 375-377.

Beadle, C. L., McLeod, D. E., Turnbull, C. R. A., Ratkowsky, D. A. and McLeod, R. (1989). Juvenile/total foliage ratios in *Eucalyptus nitens* and the growth of stands and individual trees. *Trees* 3: 117-124.

Beck, S. D. (1968). Insect Photoperiodism. Academic Press, New York. 288 pp.

Beers, E. H. and Brunner, J. F. (1992). Implementation of the codling moth phenology model on apples in Washington State USA. *Acta Phytopathologica et Entomologica Hungarica* 27: 97-102.

Begon, M., Harper, J. L. and Townsend, C. R. (1986). Ecology, Individuals, Populations and Communities. Blackwell Scientific Publications, Oxford. 876 pp.

Berry, I. L., Kunz, S. E. and Foerster, K. W. (1977). A dynamic model of the physiological development of immature stable flies. *Ann. Entomol. Soc. Am.* 70: 173-176.

Berrymann, A. A. (1986). Forest Insects: Principles and Practice of population management. Plenum Press, New York. 729 pp.

Bradford, M. J. and Roff, D. A. (1997). An empirical model of diapause strategies of the cricket *Allonemobius socius*. *Ecology* 78(2): 442-451.

Bradshaw, W. E. (1986). Pervasive themes in insect life cycle strategies. In: Taylor, F. and Karban, R. (Eds.) The Evolution of Insect Life Cycles. Springer-Verlag, New York. pp. 261-275.

Brooker, M. I. H. and Kleinig, D. A. (1990). Field Guide to Eucalypts. Revised Ed. Vol. 1. South-eastern Australia. Inkata Press, Melbourne. 288 pp.

Campbell, A., Frazer, B. D., Gilbert, N., Gutierrez, A. P. and Mackauer, M. (1974). Temperature requirements of some aphids and their parasites. *J. Appl. Ecol.* 11: 431-438.

Carne, P. B., Greaves, R. T. G. and McInnes, R. S. (1974). Insect damage to plantation-grown eucalypts in north coastal New South Wales, with particular reference to Christmas beetles (Coleoptera: Scarabaeidae). *J. Aust. Ent. Soc.* 13: 189-206.

Casagrande, R. A., Logan, P. A. and Wallner, W. E. (1987). Phenological model for gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), larvae and pupae. *Environ. Entomol.* 16: 556-562.

Common, I. F. B. (1954). A study of the ecology of the adult Bogong moth, *Agrotis infusa* (Boisd.) (Lepidoptera: Noctuidae), with special reference to its behavior during migration and aestivation. *Aust. J. Zool.* 2: 223-263.

Coulson, R. N. and Witter, J. A. (1984). Forest Entomology (Ecology and Management). John Wiley and Sons, New York. 669 pp.

Cremer, K. W. (1973). Ability of *Eucalyptus regnans* and associated evergreen hardwoods to recover from cutting or complete defoliation in different seasons. *Aust. For. Res.* 6: 9-22.

Cullen, J. M. and Browning, T. O. (1978). The influence of photoperiod and temperature on the induction of diapause in pupae of *Heliothis punctigera*. *J. Insect Physiol.* 24: 595-601.

D'amico, V. and Elkinton, J. S. (1995). Rainfall effects on transmission of gypsy moth (Lepidoptera: Lymantriidae) nuclear polyhedrosis virus. *Environ. Entomol.* 24(5): 1144-1149.

Dallwitz, M. J. and Higgins, J. P. (1992). User's Guide to DEVAR. A Computer Program for Estimating Development Rate as a Function of Temperature. CSIRO Australian Division of Entomology Report 2, Canberra. 23 pp.

Danilevskii, A. S. (1961). Photoperiodism and Seasonal Development of Insects (English translation, 1965). Oliver & Boyd, Edinburgh and London. 283 pp.

Danks, H. V. (1994a). Diversity and integration of life-cycle controls in insects. In: Danks, H. V. (Ed.) Insect Life-cycle Polymorphism: Theory, Evolution and Ecological Consequences for Seasonality and Diapause Control. Kluwer Academic publishers, London. pp. 5-40.

Danks, H. V. (1994b). Insect life-cycle polymorphism: Current ideas and future prospects. In: Danks, H. V. (Ed.) Insect Life-cycle Polymorphism: Theory, Evolution and Ecological Consequences for Seasonality and Diapause Control. Kluwer Academic publishers, London. pp. 349-365.

Day, K. R. (1997). The influence of temperature on egg mortality in the budmoth *Zeiraphera diniana* (Lepidoptera: Tortricidae), and its role in determining the regional abundance of an important forest pest. *Bulletin of Entomological Research* 87: 259-264.

de Little, D. W. (1981). A study of autumn gum moth, *Mnesampela privata* Guen. (Lepidoptera: Geometridae) on *Eucalyptus nitens* plantations on North West Tasmania. Internal Report, North Forest Products : 12pp.

Denlinger, D. L. (1985). Hormonal control of diapause. In: Kerkut, G. A. and Gilbert, L. I. (Eds.) Comprehensive insect physiology, biochemistry and pharmacology. Vol. 8. Pergamon Press, Oxford. pp. 353-412.

Dingle, H. (Ed.) (1978). Evolution of Insect Migration and Diapause. Springer-Verlag, New York. 284 pp.

Dingle, H. (1986). The evolution of insect life cycle syndromes. In: Taylor, F. and Karban, R. (Eds.) The Evolution of Insect Life Cycles. Springer-Verlag, New York. pp. 187-203.

Eldridge, K., Davidson, J., Harwood, C. and vanWyk, G. (Eds.) (1993). Eucalypt Domestication and Breeding. Clarendon Press, Oxford. 288 pp.

Elliott, H. J. and Bashford, R. (1978). The life history of *Mnesampela privata* (Guen.) (Lepidoptera: Geometridae) a defoliator of young eucalypts. *J. Aust. Ent. Soc.* 17: 201-204.

Elliott, H. J., Bashford, R., Greener, A. and Candy, S. G. (1992). Integrated pest management of the Tasmanian *Eucalyptus* leaf beetle, *Chrysophtharta bimaculata* (Olivier)(Coleoptera: Chrysomelidae). *For. Ecol. Manage.* 53: 29-38.

Farrow, R. A. (1996). Insect pests of eucalypts on farms and in plantations. CSIRO Identification Leaflet No. 1. CSIRO, Australia. 4 pp.

Farrow, R. A., Floyd, R. B. and Neumann, F. G. (1994). Inter-provenance variation in resistance of *Eucalyptus globulus* juvenile foliage to insect feeding. *Aust. For.* 57: 65-68.

Feeny, P. (1970). Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology* 51: 565-581.

Floyd, R. B. and Farrow, R. A. (1994). The potential role of natural insect resistance in the integrated pest management of eucalypt plantations in Australia. In: Halos, S. C. (Ed.) Proceedings of the symposium on biotechnological and environmental approaches to forest pest management. Quezon City, Philippines 28-30 April 1993. Forest Pest and Disease Management. Seameo Biotrop, Bogor. pp. 55-76.

Floyd, R. B., Farrow, R. A. and Neumann, F. G. (1994). Inter- and intra-provenance variation in resistance of red gum foliage to insect feeding. *Aust. For.* 57: 45-48.

Forestry Commission Tasmania (1977). Autumn Gum Moth. Forest pests and diseases Leaflet No. 2: 3pp.

French, C. (Ed.) (1900). Handbook of the Destructive Insects of Victoria, pt.3. Government Printer, Melbourne. 229 pp.

Froggatt, W. W. (Ed.) (1923). Forest Insects of Australia. Government Printer, Sydney. 171 pp.

Gauld, I. D. (1984). An Introduction to the Ichneumonidae of Australia. British Museum, London. 413 pp.

Gay, F. J. (Ed.) (1966). CSIRO Bulletin No. 285. Scientific and common names of insects and allied forms occurring in Australia. CSIRO, Australia, Melbourne. pp.

Genstat 5 Committee (1993). Genstat 5 Release 3 Reference Manual. Oxford University Press, New York. 796 pp.

Gilbert, N. (1984). Control of fecundity in *Pieris rapae* II. Differential effects of temperature. *J. Anim. Ecol.* 53: 589-597.

Gilbert, N. (1988). Control of fecundity in *Pieris rapae* V. Comparisons between populations. *J. Anim. Ecol.* 57: 395-410.

Gilbert, N. and Raworth, D. A. (1996). Insects and temperature - A general theory. *The Canadian Entomologist* 128: 1-13.

Gillott, C. (Ed.) (1995). Entomology. 2nd Ed. Plenum Press, New York. 798 pp.

Gomi, T. and Takeda, M. (1996). Changes in life-history traits in the fall webworm within half a century of introduction to Japan. *Functional Ecology* 10: 384-389.

Gordon, H. T. (1984). Growth and development of insects. In: Huffaker, C. B. and Rabb, R. L. (Eds.) Ecological Entomology. John Wiley and Sons, New York. pp. 53-77.

Grüner, C. and Masaki, S. (1994). Summer diapause in the polymorphic life cycle of the noctuid moth *Mamestra brassicae*. In: Danks, H. V. (Ed.) Insect Life-cycle Polymorphism: Theory, Evolution and Ecological Consequences for Seasonality and Diapause Control. Kluwer Academic publishers, London. pp. 191-204.

Grüner, C. and Sauer, K. P. (1988). Aestival dormancy in the cabbage moth *Mamestra brassicae* L. (Lepidoptera: Noctuidae). 1. Adaptive significance of variability of two traits: day length thresholds triggering aestival dormancy and duration of aestival dormancy. *Oecologia* 74:4: 515-523.

Holliday, N. J. (1983). Effects of temperature on winter moth pupae, *Operophtera brumata* (Lepidoptera: Geometridae). The Canadian Entomologist 115: 243-249.

Holliday, N. J. (1985). Maintenance of the phenology of the winter moth (Lepidoptera: Geometridae). *Biological Journal of the Linnean Society* 25: 221-234.

Howe, R. W. (1967). Temperature effects on embryonic development in insects. *Annu. Rev. Entomol.* 12: 15-42.

Johnsen, S., Gutierrez, A. P. and Jorgensen, J. (1997). Overwintering in the cabbage root fly *Delia radicum*: a dynamic model of temperature-dependent dormancy and post-dormancy development. *J. Appl. Ecol.* 34: 21-28.

Johnson, P. C., Mason, D. P., Radke, S. L. and Tracewski, K. T. (1983). Gypsy moth, *Lymantria dispar* (L.)(Lepidoptera: Lymantriidae), egg eclosion: Degree-day accumulation. *Environ. Entomol.* 12: 928-932.

Kaitaniemi, P., Ruohomäki, D. and Haukioja, E. (1997). Consequences of defoliation on phenological interaction between *Epirrita autumnata* and its host plant, Mountain Birch. *Functional Ecology* 11: 199-208.

Kimberling, D. N. and Miller, J. C. (1988). Effects of temperature on larval eclosion of the winter moth, *Operophtera brumata. Entomol. Exptl. Appl.* 47: 249-254.

Knapp, R. and Casey, T. M. (1986). Thermal ecology, behavior, and growth of gypsy moth (*Lymantria dispar*) and eastern tent (*Malacosoma americanum*) caterpillars. *Ecology* 67: 598-608.

Lance, D. R., Elkinton, J. S. and Schwalbe, C. P. (1987). Microhabitat and temperature effects explain accelerated development during outbreaks of the gypsy moth (Lepidoptera: Lymantriidae). *Environ. Entomol.* 16: 202-205.

Logan, J. A., Wollkind, D. J., Hoyt, S. C. and Tanigoshi, L. K. (1976). An analytic model for description of temperature dependent rate phenomena in arthropods. *Environ. Entomol.* 5(6): 1133-1140.

Logan, J. A., Casagrande, R. A. and Liebhold, A. M. (1991). Modeling environment for simulation of gypsy moth (Lepidoptera: Lymantriidae) larval phenology. *Environ. Entomol.* 20: 1516-1525.

Lutz, P. E. (1968). Effects of temperature and photoperiod on larval development in *Lestes eurinus* (Odonata: Lestidae). *Ecology* 49: 637-644.

Madden, J. L. and Bashford, R. (1977a). The life history and behaviour of *Chlenias* sp., a geometrid defoliator of radiata pine in Tasmania. *J. Aust. Ent. Soc.* 16: 371-378.

Madden, J. L. and Bashford, R. (1977b). Population biology of *Chlenias* sp., a geometrid defoliator of *Pinus radiata* in Tasmania. *J. Aust. Ent. Soc.* 16: 379-388.

Manel, S. and Debouzie, D. (1997). Modeling insect development time of two or more larval stages in the field under variable temperatures. *Environ. Entomol.* 26(2): 163-169.

Mascord, R. (1980). Spiders of Australia. Reed, Sydney. 128 pp.

McQuillan, P. B. (1981). A review of the Australian moth genus *Thalaina* (Lepidoptera: Geometridae: Ennominae). *Trans. R. Soc. S. Aust.* 105: 1-23.

McQuillan, P. B. (1985). A taxonomic revision of the Australian autumn gum moth genus *Mnesampela* Guest (Lepidoptera: Geometridae, Ennominae). *Ent. Scand.* 16: 175-202.

McQuillan, P. B. and Edwards, E. D. (1996). Geometroidea. In: Nielsen, E. S., Edwards, E. D. and Rangsi, T. V. (Eds.) Monographs on Australian Lepidoptera. Volume 4. Checklist of the Lepidoptera of Australia. CSIRO Australia, Collingwood. pp. 200-228.

McQuillan, P. B., Taylor, R. J., Bereton, R. N. and Cale, P. G. (1998). Seasonal patterns of activity in geometrid moths (Lepidoptera: Geometridae) from a lowland and highland eucalypt forest in Tasmania. *Australian Journal of Entomology* 37: 228-237.

Mendenhall, W. (1983). Introduction to Probability and Statistics. Duxbury Press, Boston. 646 pp.

Miller, J. C. (1996). Temperature-dependent development of *Meteorus communis* (Hymenoptera: Braconidae), a parasitoid of the variegated cutworm (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 89(4): 877-880.

Moran, N. (1992). The evolutionary maintenance of alternative phenotypes. Am. Nat. 139(5): 971-989.

Naumann, I. D. (1991). Hymenoptera. In: CSIRO Division of Entomology (Ed.) The Insects of Australia. 2nd ed. Vol. 2. Melbourne University Press, Melbourne. pp. 916-1000.

Neumann, F. G. (1993). Insect pest problems of eucalypt plantations in Australia. 3. Victoria. *Aust. For.* 56(4): 370-374.

Neumann, F. G. and Collett, N. G. (1997). Insecticide trials for control of the Autumn Gum Moth (*Mnesampela privata*), a primary defoliator in commercial eucalypt plantations prior to canopy closure. *Aust. For.* 60: 130-137.

Nielsen, E. S. and Common, I. F. B. (1991). Lepidoptera. In: Division of Entomology, C. S. I. R. O. (Ed.) The Insects of Australia. 2nd ed. Vol. II. Melbourne University Press, Melbourne. pp. 817-915.

Norris, K. R. (1991). General Biology. In: CSIRO Division of Entomology (Ed.) The Insects of Australia. 2nd ed. Vol. 2. Melbourne University Press, Melbourne. pp. 68-108.

Nylin, S. (1994). Seasonal plasticity and life-cycle adaptations in butterflies. In: Danks, H. V. (Ed.) Insect Life-cycle Polymorphism: Theory, Evolution and Ecological Consequences for Seasonality and Diapause Control. Kluwer Academic publishers, London. pp. 41-67.

Nylin, S. and Gotthard, K. (1998). Plasticity in life-history traits. Annu. Rev. Entomol. 43: 63-83.

Ohmart, C. P. (1990). Insect pests in intensively-managed eucalypt plantations in Australia: Some thoughts on this challenge to a new era in forest management. *Aust. For.* 53(1): 7-12.

Oku, T. (1983). Aestivation and migration in noctuid moths. In: Brown, V. K. and Hodek, I. (Eds.) Diapause and Life Cycle Strategies in Insects. Dr. W. Junk Publishers The Hague, Boston. pp. 219-231.

Olaye, A. C., Schulthess, F., Shanower, T. G. and Bosque-Perez, N. A. (1997). Factors influencing the developmental rates and reproductive potentials of *Telenomus busseolae* (Gahan) (Hym.: Scelionidae), an egg parasitoid of *Sesamia calamistis* Hampson (Lep.: Noctuidae). *Biological Control* 8: 15-21.

Pedigo, L. P. (1989). Entomology and Pest Management. Macmillan Publishing Company, New York. 646 pp.

Peterson, M. A. (1995). Phenological isolation, gene flow and developmental differences among low- and high-elevation populations of *Euphilotes enoptes* (Lepidoptera: Lycaenidae). *Evolution* 49(3): 446-455.

Peterson, N. A. and Nilssen, A. C. (1996). Nonlinear temperature-dependent development of autumnal moth pupae, *Epirrita autumnata* (Lepidoptera: Geometridae). *J. Econ. Entomol.* 89(1): 147-154.

Phillips, C. L. (1993). Insect pest problems of eucalypt plantations in Australia. 5. South Australia. *Aust. For.* 56(4): 378-380.

Phillips, C. (1996). Insects, Diseases and Deficiencies Associated With Eucalypts in South Australia. Primary Industries, South Australia. 160 pp.

Potvin, C. (1993). ANOVA: Experiments in Controlled Environments. In: Scheiner, S. M. and Gurevitch, J. (Eds.) Design and analysis of ecological experiments. Chapman and Hall, New York. pp. 46-68.

Price, P. W. (1994). Phylogenetic constraints, adaptive syndromes, and emergent properties: from individuals to population dynamics. *Res. Popul. Ecol.* 36: 3-14.

Price, P. W., Cobb, N., Craig, T. P., Fernandes, G. W., Itami, J. K., Mopper, S. and Preszler, R. W. (1990). Insect herbivore population dynamics on trees and shrubs: new approaches relevant to latent and eruptive species and life table development. In: Bernays, E. A. (Ed.) Insect-plant interactions. Vol. 2. CRC Press, Boca Raton,FL. pp. 1-38.

Pryor, L. D. and Johnson, L. A. S. (1971). A Classification of the Eucalypts. Australian National University Press, Canberra. 102 pp.

Read, N. R. and Moon, R. D. (1996). Simulation of development and survival of *Aedes vexans* (Diptera: Culicidae) larvae and pupae. *Environ. Entomol.* 25(5): 1113-1121.

Riedl, H. (1983). Analysis of codling moth phenology in relation to latitude, climate and food availability. In: Brown, V. K. and Hodek, I. (Eds.) Diapause and Life Cycle Strategies in Insects. Dr. W. Junk Publishers The Hague, Boston. pp. 233-252.

Roberts, R. J. and Sawtell, N. L. (1981). Survival and growth of local and other eucalypts planted on the northern tablelands. In: Old, K. M., Kile, G. A. and Ohmart, C. P. (Eds.) Eucalypt Dieback in Forests and Woodlands. CSIRO, Melbourne. pp. 87-94.

Roland, J. and Kaupp, W. J. (1995). Reduced transmission of forest tent caterpillar (Lepidoptera: Lasiocampidae) nuclear polyhedrosis virus at the forest edge. *Environ. Entomol.* 24(5): 1175-1178.

Sauer, K. P., Spieth, H. and Grüner, C. (1986). Adaptive significance of genetic variability of photoperiodism in Mecoptera and Lepidoptera. In: Taylor, F. and Karban, R. (Eds.) The Evolution of Insect Life Cycles. Springer-Verlag, New York. pp. 153-172.

Saunders, D. S. (1976). Insect Clocks. Pergamon Press, Oxford. 280 pp.

Schneider, S. H. (1992). The climatic response to greenhouse gases. *Advances in Ecological Research* 22: 1-32.

Scoble, M. J. (1992). The Lepidoptera, Form, Function and Diversity. Oxford University Press, New York. 404 pp.

Shepherd, R. F. (1994). Management strategies for forest insect defoliators in British Columbia. *For. Ecol. Manage.* 68: 303-324.

Spurgeon, D. W., Lingren, P. D., Raulston, J. R. and Shaver, T. N. (1995). Pupal development and adult emergence patterns of the Mexican Rice Borer (Lepidoptera: Pyralidae). *Environ. Entomol.* 24(1): 76-79.

Takeda, M. and Skopik, S. D. (1997). Photoperiodic time measurement and related physiological mechanisms in insects and mites. *Annu. Rev. Entomol.* 42: 323-349.

Tammaru, T., Kaitaniemi, P. and Ruohomäki, K. (1995). Oviposition choices of *Epirrita autumnata* (Lepidoptera: Geometridae) in relation to its eruptive population dynamics. Oikos 74: 296-304.

Tammaru, T., Kaitaniemi, P. and Ruohomaki, K. (1996). Realized fecundity in *Epirrita autumnata* (Lepidoptera: Geometridae): relation to body size and consequences to population dynamics. *Oikos* 77: 407-416.

Tauber, M. J. and Tauber, C. A. (1978). Evolution of phenological strategies in insects: A comparative approach with eco-physiological and genetic considerations. In: Dingle, H. (Ed.) Evolution of insect migration and diapause. Springer-Verlag, New York. pp. 53-71.

Tauber, M. J., Tauber, C. A. and Masaki, S. (1984). Adaptations to hazardous seasonal conditions: Dormancy, migration, and polyphenism. In: Huffaker, C. B. and Rabb, R. L. (Eds.) Ecological Entomology. John Wiley and Sons, New York. pp. 149-183.

Taylor, F. (1986). Toward a theory for the evolution of the timing of hibernal diapause. In: Taylor, F. and Karban, R. (Eds.) The Evolution of Insect Life Cycles. Springer-Verlag, New York. pp. 236-257.

Thornhill, R. and Alcock, J. (1983). The Evolution of Insect Mating Systems. Harvard University Press, Cambridge. 547 pp.

Tibbits, W. N. (1986). Eucalypt plantations in Tasmania. *Aust. For.* 49: 219-225.

Topp, W. (1994). Seasonal time partitioning and polymorphism in the developmental cycles of sympatric *Staphylinoidea* (Coleoptera) living in an unstable environment. In: Danks, H. V. (Ed.) Insect Life-cycle Polymorphism: Theory, Evolution and Ecological Consequences for Seasonality and Diapause Control. Kluwer Academic publishers, London. pp. 277-312.

Topp, W. and Kirsten, K. (1991). Synchronisation of pre-imaginal development and reqroductive success in the winter moth, *Operophtera brumata* L. *J. Appl. Ent.* 111: 137-146.

Van Dongen, S., Backeljau, T., Matthysen, E. and Dhondt, A. A. (1997). Synchronization of hatching date with budburst of individual host trees (*Quercus robur*) in the winter moth (*Operophtera brumata*) and its fitness consequences. *J. Anim. Ecol.* 66: 113-121.

Via, S. (1994). The evolution of phenotypic plasticity: What do we really know? In: Real, L. A. (Ed.) Ecological Genetics. Princeton University Press, Princeton, New Jersey. pp. 35-57.

Waldbauer, G. P. (1978). Phenological adaptation and the polymodal emergence patterns of insects. In: Dingle, H. (Ed.) Evolution of Insect Migration and Diapause. Springer-Verlag, New York. pp. 127-144.

Wardhaugh, K. G. (1986). Diapause strategies in the Australian plague locust (*Chortoicetes terminifera* Walker). In: Taylor, F. and Karban, R. (Eds.) The Evolution of Insect Life Cycles. Springer-Verlag, New York. pp. 89-104.

Watt, T. A. (1993). Introductory Statistics for Biology Students. Chapman & Hall, London. 185 pp.

Williams, M. D., Beadle, C. L., Turnbull, C. R. A., Dean, G. H. and French, J. (1995). Papermaking potential of plantation eucalypts. In: Potts, B. M., Borralho, N. M. G., Reid, J. B., Cromer, R. N., Tibbits, W. N. and Raymond, C. A. (Eds.) 'Eucalypt Plantations: Improving Fibre Yield and Quality'. Proc. CRCTHF - IUFRO Conf., Hobart, 19-24 Feb. CRC for Temperate Hardwood Forestry, Hobart. pp. 73-78.

Winston, P. W. and Bates, D. H. (1960). Saturated solutions for the control of humidity in biological research. *Ecology* 41(1): 232-237.

Wylie, F. R. and Peters, B. C. (1993). Insect pest problems of eucalypt plantations in Australia. 1. Queensland. *Aust. For.* 56(4): 358-362.

Wylie, H. G. (1960). Some factors that affect the annual cycle of the winter moth, *Operophtera brumata* in western Europe. *Entomol. Exptl. Appl.* 3: 93-102.

Zalucki, M. P., Daglish, G., Firempong, S. and Twine, P. (1986). The biology and ecology of *Heliothis armigera* (Hübner) and *H. punctigera* Wallengren (Lepidoptera: Noctuidae) in Australia: What do we know? *Aust. J. Zool.* 34: 779-814.

Zalucki, M. P., Murray, D. A. H., Gregg, P. C., Fitt, G. P., Twine, P. H. and Jones C. (1994). Ecology of *Helicoverpa armigera* (Hübner) and *H. punctigera* (Wallengren) in the inland of Australia: Larval sampling and host plant relationships during winter and spring. *Aust. J. Zool.* 42: 329-346.

Zeiss, M. R., Koehler, K. J. and Pedigo, L. P. (1996). Degree-day requirements for development of the bean leaf beetle (Coleoptera: Chrysomelidae) under two rearing regimes. *J. Econ. Entomol.* 89(1): 111-118.

APPENDIX A

NATURAL ENEMIES OF *M. PRIVATA*

The immature stages of *M. privata* were known to be attacked by a range of natural enemies (Table A-1). Eggs of *M. privata* are parasitized by *Telenomus* sp. (Hymenoptera: Scelionidae) (Elliott & Bashford, 1978; de Little, 1981), while larvae are parasitized by several 'koinobiont' parasitoids, i.e. species which allow the host to continue growth and development after initial parasitization, but ultimately kill the host (Naumann, 1991). Seven species of ichneumonid, one braconid and one tachinid have been previously recorded as primary parasitoids from *M. privata* (Table A-1). Young larvae are also attacked by spiders and insect predators such as *Oechalia schellembergii* (Guérin-Méneville) (Hemiptera: Pentatomidae) (Farrow, 1996) and an unidentified myrid (Hemiptera: Myridae) (de Little, 1981). Although natural enemies of *M. privata* were not the focus of this thesis, information was gathered on the egg parasitoid *Telenomus* sp. and the two ichneumonids, *Anacis* sp. (identified by I. Naumann, CSIRO Entomology) and *Heteropelma scaposum* (Morley).

A.1 EGG PARASITISM

A.1.1 Introduction

Eggs of *M. privata* are parasitized by *Telenomus* in Tasmania (Elliott & Bashford, 1978; de Little, 1981) and on mainland Australia (Schumacher, unpubl.) although it is not known whether this is the one species across the collection region. The rate of egg parasitism by *Telenomus* is generally low, with Elliott & Bashford (1978) recording an overall rate of 2.57% (1596 eggs) in southern Tasmania, while de Little (1981) found mean parasitization rates of 19.3% and 6.7% at two sites in NW Tasmania.

Table A- 1 List of known predators and parasitoids of *Mnesampela privata*.

Stage	Genus	Biology	Locality	Order	Family	Stage Attacked	Stage Killed	Source [a]
	Predators							
1st instar larvae	Unidentified myrid bug [b]	Solitary	NW Tas.	Hemiptera	Myridae	egg, larva		D
Young larvae	*Oechalia schellembergii* (Guérin-Méneville)	Solitary	Mainland	Hemiptera	Pentatomidae	larva		F
	Parasitoids							
Eggs	*Telenomus* sp.	Solitary	Tasmania.+ Mainland	Hymenoptera	Scelionidae	egg	egg	EB, D
Larvae	*Apanteles* sp.	Gregarious	Mainland	Hymenoptera	Braconidae	larva	larva	S
	Campoplex sp.	Solitary	SE Tas.	Hymenoptera	Ichneumonidae	larva	larva	EB
	Casinaria sp.	Solitary	SE Tas.	Hymenoptera	Ichneumonidae	larva	larva	EB, G
	Eriborus sp.	Solitary	SE Tas.	Hymenoptera	Ichneumonidae	larva	larva	EB, G
	Megaceria sp.	not specified	not specified	Hymenoptera	Ichneumonidae	larva	larva	G
	Pristiceros sp.	not specified	not specified	Hymenoptera	Ichneumonidae	larva	larva	G
	Anacis sp. [c]	Solitary	NW Tas.	Hymenoptera	Ichneumonidae	larva	pupa	D
	Heteropelma scaposum (Morley)	Solitary	NW Tas.+ Mainland	Hymenoptera	Ichneumonidae	larva	pupa	D,F
	Unidentified tachinid	Solitary	Tasmania	Diptera	Tachinidae	larva	pupa	EB, D

[a] D = de Little (1981); EB = Elliott & Bashford, (1978); F = Farrow (1996); G = Gauld (1984); S = Schumacher (unpubl. data)
[b] The author has observed one species of myrid bug from NW Tasmania feeding on developing *M. privata* eggs as well as on first instar larvae. Myrids confined to petrie dishes with neonate *M. privata* larvae began cannibalising each other after they destroyed all larvae.
[c] Identified by I. Naumann, CSIRO Entomology, Canberra.

In a preliminary study assessing the potential use of *Telenomus* as a biological control agent against *M. privata*, Schumacher (unpublished data) reared two field-parasitized egg masses collected nine and 52 days after deposition in the field for a further 35 and 17 days respectively at 20°C before adult parasitoids emerged. Since Schumacher also found that the total development time required by *M. privata* eggs to hatch at 20°C was 12 days, her results indicate the total developmental period of *Telenomus* is significantly longer than that for its host eggs. Effective natural enemies generally have a short generation time in relation to that of the host stage attacked and are able to show a rapid numerical response to increasing host populations (Berryman, 1986). Hence, a better understanding of the thermal requirements of *Telenomus* is needed to determine whether *Telenomus* can complete more than one generation during its host's oviposition period in the field. An experiment was therefore set up to determine the total developmental time required by *Telenomus* to develop to an adult in *M. privata* eggs.

A.1.2 Methods

Parasitized egg batches of *M. privata* (as determined by their black colour) were collected from the Wages Rd. plantation on 2 Aug, 1995 (winter) and subsequently incubated to adult wasp emergence at constant temperatures of 10, 15, 20 and 25°C in the laboratory. Five batches of approximately 30 parasitized eggs were reared at each temperature. Each batch of 30 eggs was held in a separate, small glass vial covered with fine mesh to prevent emerging adult wasps from escaping. The five vials at each temperature were held in a transparent, cylindrical plastic container and covered with damp paper towel to reduce disiccation of eggs. All egg batches were checked daily for wasp emergence. Emerged wasps were removed from vials, immersed in 70% ethanol and then counted. Voucher specimens have been sent to

the Australian National Insect Collection (ANIC) at CSIRO Entomology Canberra for comparison with specimens reared from eggs by Schumacher.

The median number of days required for adult eclosion from host eggs was recorded at each temperature. The thermal constant (number of degree-days) and lower thermal threshold required by the parasitoid to complete development from the collection date were estimated by linear regression as described previously in Section 2.5.

A.1.3 Results

The median time taken for *Telenomus* adults to eclose from parasitized eggs was inversely related to temperature, ranging from 13 d at 25°C (n = 78) to 124 d at 10°C (n = 12) (Table A-2). The period at 10°C is only approximate since wasps were originally thought to have died after none emerged after two months at 10°C. However, 12 wasps were found dead in vials after 124 days, so that time was used as

Table A- 2 Median development times taken for *Telenomus sp.* adults to emerge from parasitized eggs held at seven temperature regimes.

Incubation Temperature (°C)	Median Duration (Days)	Range (Days)	No. Parasitized eggs	No. Wasps Emerged
10	124	124 [a]	150	12
15	36	11-42	150	21
20	19	16-27	150	75
25	13	11-16	150	78

[a] Wasps were originally thought to have died at 10°C, but 12 were found dead in vials after 124 days, which was used as the estimated development time.

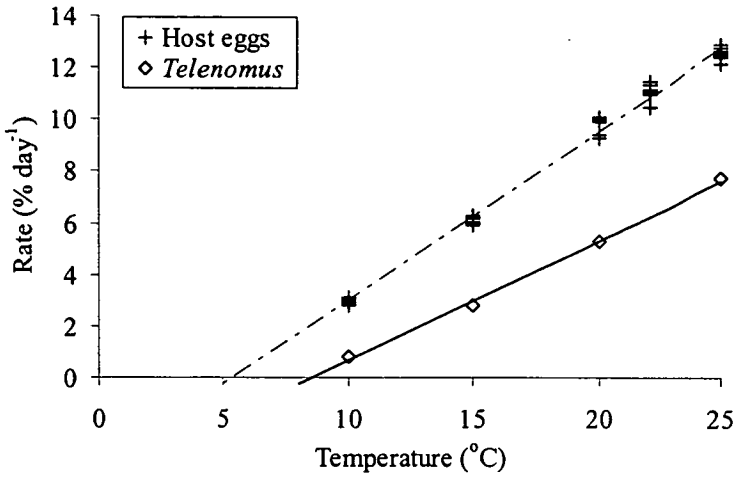


Figure A- 1 Linear relationship between temperature and the development of *Telenomus* sp. egg parasitoids of *Mnesampela privata*.

the estimated development time. Assuming a development time of 124 days at 10°C, the relationship between *Telenomus* development rate and temperature was strongly linear between 10 and 25°C (Fig. 9.1) ($F_{1,2} = 780.05$; $r^2 = 0.9974$; $p < 0.01$) and is summarized by the equation $R = 0.46\ (T - 8.57)$, where R = development rate in percent per day and T = the incubation temperature in °C. On the basis of this result a lower thermal threshold of 8.57°C and a thermal constant of 216 DD were estimated for *Telenomus* to complete development after initial collection in August.

In October 1996, newly laid *M. privata* eggs (laid by a virgin female moth) were exposed to newly emerged parasitoids and subsequently incubated at a fluctuating temperature regime of 24/18°C 12L:12D. Total developmental time (initial parasitization to adult emergence) of wasps at that regime was 24 days (n=10). Using a developmental threshold of 8.57°C from the previous year's regression results, 298.32 DD was estimated to be required by *Telenomus* sp. to complete a full generation. Extrapolation from this result predicts that 26 days would be required to complete a full generation at 20°C and contrasts with the 35

day incubation period at 20°C required by 9 day-old egg batches to yield adult parasitoids in Schumacher's (unpubl.) study. Thus, different species of *Telenomus* may be responsible for parasitism of *M. privata* eggs in Tasmania and on mainland Australia.

The regression results suggest that *Telenomus* is unlikely to develop at temperatures below 8°C. Given this situation, development of the parasitoid would be restricted to the six warmest months of the year (November to April) at high altitude sites in Tasmania, e.g. Surrey Hills and Tarraleah. Since parasitized batches collected during winter developed quickly when exposed to warmer temperatures (Table A-2), *Telenomus* probably overwinters in simple thermal quiescence as partially developed larvae inside *M. privata* eggs, resuming development the following spring when mean daily temperatures first rise above its threshold of 8.57°C. The results also indicate that the Tasmanian species of *Telenomus* requires more degree-days above a higher developmental threshold to complete a full generation than *M. privata* eggs require to hatch (165 ± 5°Cd > 5.75°C; Experiment 1 in Chapter 4). Therefore, eggs of *M. privata* are capable of developing at lower temperatures than the parasitoid and will complete development earlier than *Telenomus*. Given this situation, it should not be expected that *Telenomus* will complete more than one generation during the oviposition period of its host, assuming relatively synchronous oviposition by *M. privata*.

A.2 PUPAL PARASITISM

The biology of unparasitized pupae of *M. privata* has been covered previously in Chapters 2-4. This section focuses exclusively on the relationship between *M. privata* and two species of ichneumonid parasitoids, *Anacis* sp. and *H. scaposum* (Fig. A-2), which oviposit in the larvae of their host species but do not destroy the host until after pupation. Both species of ichneumonid have been previously observed ovipositing in *M. privata* larvae in north-western Tasmania (de Little, 1981) and *H. scaposum* is known to attack *M. privata* on mainland Australia (Farrow, 1996).

In accordance with de Little (1981), both ichneumonids were observed ovipositing in *M. privata* larvae in NW Tasmania. Oviposition by *H. scaposum* was only observed in first-instar larvae – none was observed in later instars. In contrast, *Anacis* sp. was usually seen walking over foliage and crawling into larval shelters in search of older larvae (Fig. A-3). It readily oviposited in 5th instar larvae it found exposed on the leaf surface inside thin silk shelters. Both parasitoid species were observed in central and southern Tasmania and also reared out of pupae following larval collection in Victoria, suggesting that they may be widely distributed in south-eastern Australia.

Like rates of egg parasitism by *Telenomus*, parasitism rates by these species are generally low, with de Little (1981) estimating an overall rate of 10% in NW Tasmania from larval dissections (1061 larvae). Parasitism rates by these species are presented here since no one has yet reported rearing these parasitoids directly from *M. privata* pupae. The information was gathered as a consequence of this study's focus on the pupal stage of *M. privata*, which the parasitoids ultimately destroy in order to complete their own life-cycles.

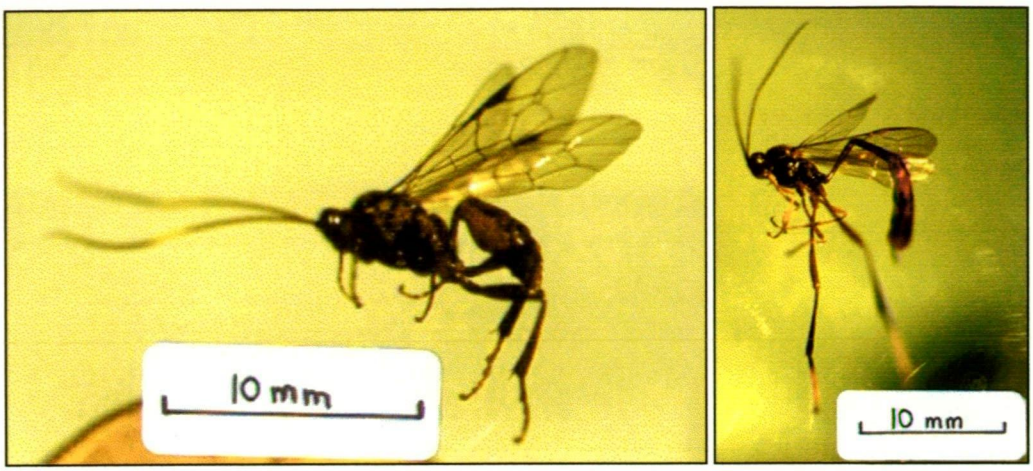


Figure A- 2 Two larval-pupal parasitoids of *Mnesampela privata*, both belonging to the family Ichneumonidae: *Anacis* sp. (left) and *Heteropelma scaposum* (right).

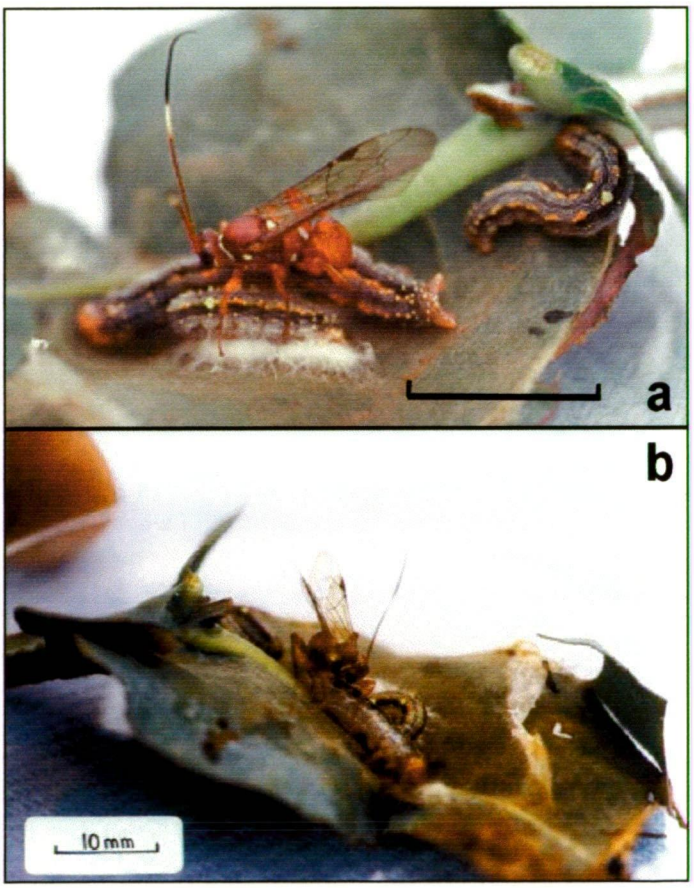


Figure A- 3 *Anacis* sp. female ovipositing in a paralysed 5th instar larva of *Mnesampela privata.* a) White silk cocoons beneath the bottom larva are from *Apanteles* sp., a gregarious braconid parasitoid that usually emerges from and pupates next to 4th and 5th instar host larvae inside leaf shelters. Scale 10mm. b) *Anacis* female concentrating its efforts on the larger larva. The two other larvae were ignored completely during the five minute period that these photographs were taken.

A.3 METHODS USED TO STUDY PUPAL PARASITISM

The fate of each pupa reared in the laboratory during the study was recorded as one of three possible outcomes: (i) a moth emerged, (ii) a parasitoid emerged or (iii) the pupa died before a moth or parasitoid emerged. Separate records were maintained for each pupal cohort, enabling seasonal and geographic variation in parasitism rates to be examined. At the end of the study the information from all cohorts was combined to examine the overall impact of parasitism on the pupal stage of *M. privata*. That information is presented below.

A.4 RESULTS

Adult parasitoids were reared from 110 of 1211 pupae that formed in the laboratory after mature larvae were collected in the field (Table A-3). This represents an overall parasitism rate of 9.1%, which is similar to the 10% larval parasitism rate estimated by de Little (1981) in N.W. Tasmania. However, parasitism rates varied between cohorts, ranging from zero to 35.4%. *Anacis* sp. was the most common parasitoid encountered, emerging from 56 pupae, followed by *H. scaposum* (n=44) and an unidentified tachinid (n=10). Both ichneumonids were reared from larvae collected in NW, central and SE Tasmania and also from larvae collected in Victoria. The tachinid was only reared from larvae collected from *E. nitens* plantations at higher altitudes in Tasmania, although Elliott & Bashford (1978) reared a tachinid from larvae collected in southern Tasmania.

Generally, adult parasitoids emerged in synchrony with adults of their host species (Fig. A-4). It appears that the development of the parasitoids is tightly linked to the development of their host. Perhaps the hormones regulating diapause and development in *M. privata* pupae are used as developmental cues by the parasitoids (see Askew, 1971).

Table A- 3 Fate of *Mnesampela privata* pupae derived from mature larvae collected in the field during the study (1995 - 1997).) OS-95.xls

Coll-date	Location	Pupae (n)	*Anacis* sp.	*H. scaposum*	Tachinid	Number Parasitized [a]	% Parasitized
1995							
19-Feb	Blythe Rd.	26	1	-	-	1	3.8
22-Mar	Wages Rd.	17	2	1	1	4	23.5
5-Apr	Wages Rd.	10	1	-	-	1	10.0
11-May	Blythe Rd.	9	-	-	-	0	0
12-Jul	Blythe Rd.	12	-	-	-	0	0
13-Jul	Sorell	5	-	-	-	0	0
2-Aug	Blythe Rd.	11	1	-	-	1	9.1
11-Aug	Sorell	27	-	-	-	0	0
31-Oct	Blythe Rd.	14	5	-	-	5	35.7
1995 Total		**131**	**10**	**1**	**1**	**12**	**9.2**
1996							
15-Feb	Blythe Rd.	32	9	1	1	11	34.4
22-Feb	Sorell	14	1	-	-	1	7.1
5-8th Mar	Victoria	31	2	1	-	3	9.7
12-Mar	Sorell	6	1	1	-	2	33.3
20-March	Tarraleah	58	2	4	-	6	10.3
20-March	Islet Creek	217	11	17	3	31	14.3
2-April	Blythe Rd.	41	2	2	-	4	9.8
9-May	Blythe Rd.	51	4	5	-	9	17.6
10-May	Tarraleah	58	-	3	-	3	5.2
10-May	Islet Creek	50	1	2	1	4	8.0
June-Oct	Hobart region	281	7	-	-	7	2.5
1996 Total		**839**	**40**	**36**	**5**	**81**	**9.7**
1997							
14-March	Penna	57	-	-	-	0	0
March-May	Central Tas. [b]	48	6	7	4	17	35.4
June	Sorell	101	-	-	-	0	0
July	Victoria	35	-	-	-	0	0
1997 Total		**241**	**6**	**7**	**4**	**17**	**7.1**
Grand Total		**1211**	**56**	**44**	**10**	**110**	**9.1**

[a] Parasitism refers only to parasitoids which successfully emerged from pupae.
[b] Combined results for two plantations south of Tarraleah in central Tasmania.

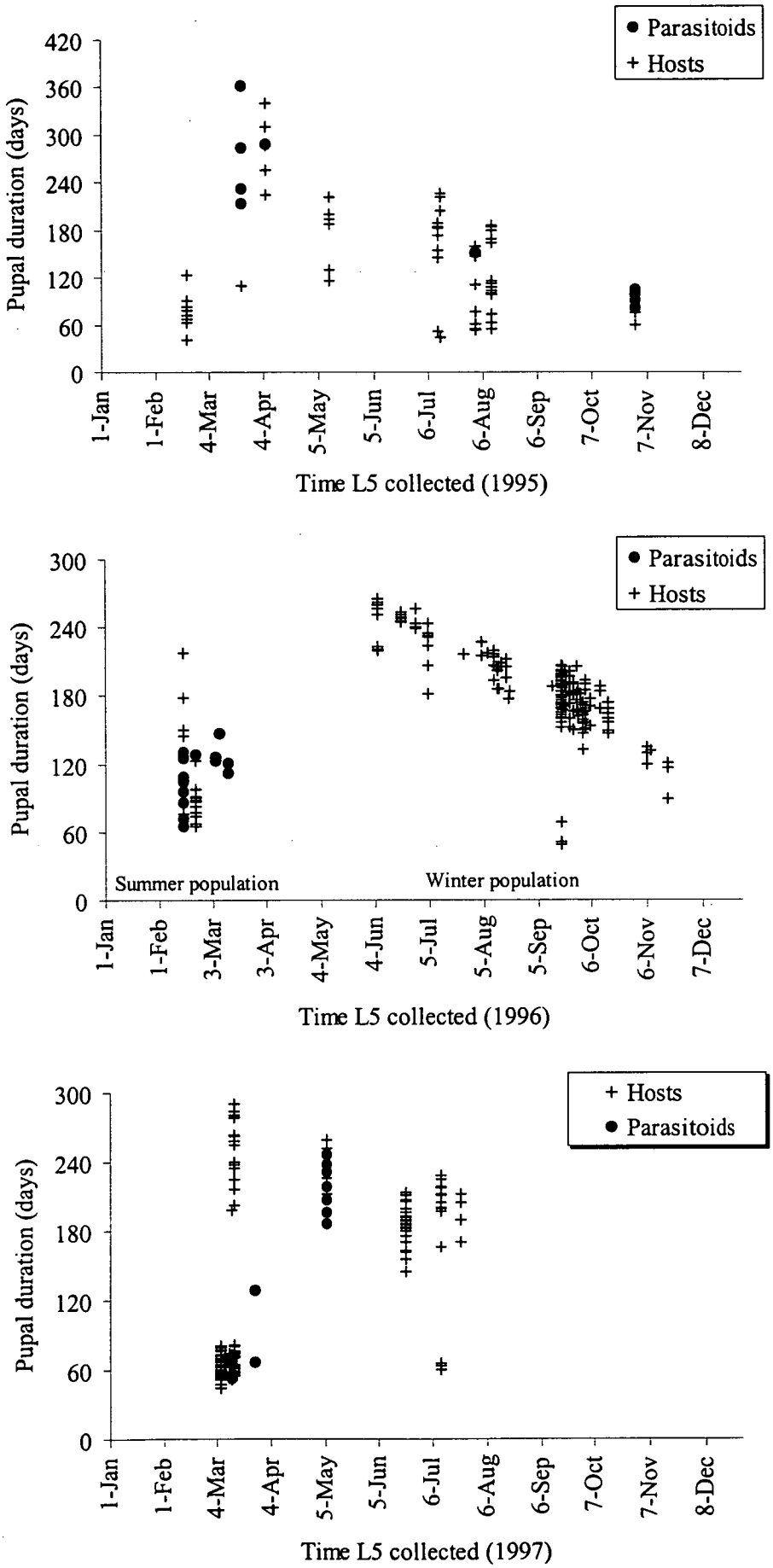


Figure A- 4 The time taken for adult parasitoids to emerge from host pupae in relation to larval collection time and host pupal duration. Parasitoid emergence usually coincided with host emergence.

Field validation. Trial-1. Egg development at SORELL.									
If minimum temperature < lower threshold, minimum temperature set at lower threshold.									
If maximum temperature > 25°C, maximum temperature set at 25°C.									
Oviposition on 7th May 1996.									
				THERMAL ACCUMULATION > 7 MAY 1996.					
DD = (min + max)/2 - threshold				DD > 5.87 °C			DD > 5.00 °C		
	Date	Min. °C	Max. °C	Daily	Total		Daily	Total	
Ovipos.	7/05/1996	5.9	14.6		0.0			0.0	
	8/05/1996	6.8	12.0	3.5	3.5		4.4	4.4	
	9/05/1996	3.8	19.2	6.7	10.2		7.1	11.5	
	10/05/1996	6.9	23.7	9.4	19.6		10.3	21.8	
	11/05/1996	11.4	13.7	6.7	26.3		7.6	29.4	
	12/05/1996	5.4	12.2	3.2	29.5		3.8	33.2	
	13/05/1996	5.1	14.7	4.4	33.9		4.9	38.1	
	14/05/1996	8.4	14.9	5.8	39.7		6.7	44.7	
	15/05/1996	12.6	15.2	8.0	47.7		8.9	53.6	
	16/05/1996	11.1	15.6	7.5	55.2		8.4	62.0	
	17/05/1996	6.7	14.9	4.9	60.1		5.8	67.8	
	18/05/1996	5.0	10.7	2.4	62.5		2.9	70.6	
	19/05/1996	7.0	14.0	4.6	67.2		5.5	76.1	
	20/05/1996	4.4	12.9	3.5	70.7		4.0	80.1	
	21/05/1996	6.0	15.8	5.0	75.7		5.9	86.0	
	22/05/1996	10.1	15.7	7.0	82.7		7.9	93.9	
	23/05/1996	10.1	15.7	7.0	89.8		7.9	101.8	
	24/05/1996	11.0	14.1	6.7	96.4		7.6	109.3	
	25/05/1996	10.9	15.1	7.1	103.6		8.0	117.3	
	26/05/1996	5.9	15.0	4.6	108.1		5.5	122.8	
	27/05/1996	5.7	15.0	4.6	112.7		5.4	128.1	
	28/05/1996	8.6	14.0	5.4	118.1		6.3	134.4	
	29/05/1996	9.0	15.8	6.5	124.7		7.4	141.8	
	30/05/1996	10.6	17.2	8.0	132.7		8.9	150.7	
	31/05/1996	13.1	15.4	8.4	141.1		9.3	160.0	
	1/06/1996	6.9	16.7	5.9	147.0		6.8	166.8	
	2/06/1996	7.6	15.4	5.6	152.6		6.5	173.3	pred-L1
	3/06/1996	5.0	15.3	4.7	157.4		5.2	178.4	
	4/06/1996	5.6	10.9	2.5	159.9		3.3	181.7	
L1-hatch	5/06/1996	7.1	16.5	5.9	165.8	pred-L1	6.8	188.5	
THRESHOLDS AND DD FROM TABLE 3.11.									
1. Using egg threshold of 5.87 °C, Predicted hatch = 160.95 DD > 5.87 °C.									
Predicted hatch date = 5 June 1996.									
Observed hatch date = 5 June 1996.									
Observed hatch was 0 days later at 165.80 DD (3.0% more than predicted).									
2. Using fixed threshold of 5.00 °C, Predicted hatch = 171.29 DD > 5.00 °C.									
Predicted hatch date (pred-L1) = 2 June 1996.									
Observed hatch date (L1-hatch) = 5 June 1996.									
Observed hatch was 3 days later at 188.45 DD (10.0% more than predicted).									

Field validation. Trial-2 at CRC. Eggs and larvae reared outside.									
If minimum temperature < lower threshold, minimum temperature set at lower threshold.									
If maximum temperature > 25°C, maximum temperature set at 25°C.									
Oviposition on 22nd June 1996.				THERMAL ACCUMULATION > 22 JUNE 1996.					
DD = (min + max)/2 - threshold				DD>5 °C		DD> DZ for each instar			
				Daily	Total	Daily	Total		
Ovipos.	22/06/1996	8.6	10.5		0.0		0.0	Eggs	
	23/06/1996	4.4	11.2	3.1	3.1	2.7	2.7	DZ= 5.87	
	24/06/1996	6.1	12.6	4.4	7.5	3.5	6.1		
	25/06/1996	6.3	13.2	4.8	12.2	3.9	10.0		
	26/06/1996	5.0	13.2	4.1	16.3	3.7	13.7		
	27/06/1996	5.8	9.5	2.7	19.0	1.8	15.5		
	28/06/1996	3.2	11.1	3.1	22.0	2.6	18.1		
	29/06/1996	1.2	8.0	1.5	23.5	1.1	19.2		
	30/06/1996	-0.3	8.3	1.7	25.2	1.2	20.4		
	1/07/1996	0.9	11.9	3.5	28.6	3.0	23.4		
	2/07/1996	5.0	12.6	3.8	32.4	3.4	26.8		
	3/07/1996	6.6	14.1	5.4	37.8	4.5	31.3		
	4/07/1996	3.7	13.6	4.3	42.1	3.9	35.1		
	5/07/1996	5.4	12.0	3.7	45.8	3.1	38.2		
	6/07/1996	6.7	12.0	4.4	50.1	3.5	41.7		
	7/07/1996	6.5	9.4	3.0	53.1	2.1	43.8		
	8/07/1996	4.2	8.7	1.9	54.9	1.4	45.2		
	9/07/1996	3.6	6.1	0.6	55.5	0.1	45.3		
	10/07/1996	3.9	9.0	2.0	57.5	1.6	46.8		
	11/07/1996	5.9	10.3	3.1	60.6	2.2	49.1		
	12/07/1996	6.0	9.8	2.9	63.5	2.0	51.1		
	13/07/1996	7.7	10.0	3.9	67.3	3.0	54.1		
	14/07/1996	6.6	10.4	3.5	70.8	2.6	56.7		
	15/07/1996	6.1	10.1	3.1	73.9	2.2	58.9		
	16/07/1996	4.2	10.0	2.5	76.4	2.1	61.0		
	17/07/1996	1.9	10.3	2.7	79.1	2.2	63.2		
	18/07/1996	3.0	15.0	5.0	84.1	4.6	67.8		
	19/07/1996	4.9	15.5	5.3	89.3	4.8	72.6		
	20/07/1996	7.2	12.4	4.8	94.1	3.9	76.5		
	21/07/1996	4.5	12.0	3.5	97.6	3.1	79.6		
	22/07/1996	5.1	15.0	5.1	102.7	4.6	84.2		
	23/07/1996	5.8	12.5	4.2	106.8	3.3	87.5		
	24/07/1996	5.0	9.6	2.3	109.1	1.9	89.3		
	25/07/1996	4.6	12.0	3.5	112.6	3.1	92.4		
	26/07/1996	5.8	15.5	5.7	118.3	4.8	97.2		
	27/07/1996	4.3	14.7	4.9	123.1	4.4	101.6		
	28/07/1996	5.6	11.0	3.3	126.4	2.6	104.2		
	29/07/1996	7.7	12.7	5.2	131.6	4.3	108.5		
	30/07/1996	10.6	14.5	7.6	139.2	6.7	115.2		
	31/07/1996	8.2	17.1	7.7	146.8	6.8	122.0		
	1/08/1996	8.5	14.2	6.4	153.2	5.5	127.5		
	2/08/1996	4.8	13.8	4.4	157.6	4.0	131.4		
	3/08/1996	8.0	13.4	5.7	163.3	4.8	136.3		
	4/08/1996	5.3	8.5	1.9	165.2	1.3	137.6		
	5/08/1996	5.0	12.6	3.8	169.0	3.4	141.0		
pred-L1	6/08/1996	6.9	15.7	6.3	175.3	5.4	146.4		
	7/08/1996	3.9	10.1	2.6	177.8	2.1	148.5		
	8/08/1996	2.7	11.5	3.3	181.1	2.8	151.3		
	9/08/1996	5.3	14.0	4.7	185.7	4.1	155.4		
	10/08/1996	4.1	13.9	4.5	190.2	4.0	159.4		
pred-L1	11/08/1996	6.2	16.6	6.4	196.6	5.5	164.9		
L1-hatch	12/08/1996	10.0	15.1	7.6	204.1	7.2	7.2	DZ= 5.32	171.6
	13/08/1996	7.3	17.2	7.3	211.4	6.9	14.2		

	14/08/1996	9.0	11.1	5.1	216.4	4.7	18.9		
	15/08/1996	3.4	14.5	4.8	221.2	4.6	23.5		
	16/08/1996	6.9	14.6	5.8	226.9	5.4	28.9		
	17/08/1996	7.5	11.3	4.4	231.3	4.1	33.0		
	18/08/1996	3.9	9.0	2.0	233.3	1.8	34.8		
	19/08/1996	1.9	12.2	3.6	236.9	3.4	38.3		
	20/08/1996	4.5	13.8	4.4	241.3	4.2	42.5		
	21/08/1996	3.6	10.5	2.8	244.1	2.6	45.1		
	22/08/1996	4.5	12.9	4.0	248.0	3.8	48.9		
	23/08/1996	6.4	15.5	6.0	254.0	5.6	54.5		
pred-L2	24/08/1996	4.7	15.7	5.4	259.3	5.2	59.7		
	25/08/1996	7.8	16.6	7.2	266.5	6.9	66.6		
	26/08/1996	8.7	15.0	6.9	273.4	6.5	73.1		
	27/08/1996	6.7	13.0	4.9	278.2	4.5	77.7		
	28/08/1996	7.0	11.0	4.0	282.2	3.7	81.3		
pred-L2	29/08/1996	5.0	14.9	5.0	287.2	4.8	86.1		
	30/08/1996	6.1	13.0	4.6	291.7	4.9	4.9	DZ= 4.63	
	31/08/1996	4.7	13.5	4.3	296.0	4.5	9.4		
	1/09/1996	4.6	13.6	4.3	300.3	4.5	13.9		
	2/09/1996	4.7	13.3	4.2	304.4	4.4	18.2		
	3/09/1996	6.1	13.0	4.6	309.0	4.9	23.2		
	4/09/1996	4.1	13.9	4.5	313.4	4.6	27.8		
	5/09/1996	6.5	13.8	5.2	318.6	5.5	33.3		
pred-L3	6/09/1996	7.2	12.2	4.7	323.3	5.1	38.4		
	7/09/1996	2.4	12.5	3.8	327.0	3.9	42.3		
	8/09/1996	7.1	12.6	4.9	331.9	5.2	47.5		
	9/09/1996	5.4	12.9	4.2	336.0	4.5	52.1		
	10/09/1996	2.2	11.2	3.1	339.1	3.3	55.4		
	11/09/1996	2.6	14.5	4.8	343.9	4.9	60.3		
pred-L3	12/09/1996	5.7	14.0	4.9	348.7	5.2	65.5		
	13/09/1996	3.4	9.4	2.2	350.9	1.8	1.8	DZ= 5.89	
	14/09/1996	2.5	14.5	4.8	355.7	4.3	6.1		
	15/09/1996	8.8	18.5	8.7	364.3	7.8	13.8		
	16/09/1996	11.7	19.1	10.4	374.7	9.5	23.3		
	17/09/1996	8.0	15.2	6.6	381.3	5.7	29.0		
	18/09/1996	6.0	18.3	7.2	388.5	6.3	35.3		
pred-L4	19/09/1996	8.7	16.3	7.5	396.0	6.6	41.9		
	20/09/1996	5.3	11.3	3.3	399.3	2.7	44.6		
	21/09/1996	1.9	15.4	5.2	404.5	4.8	49.4		
	22/09/1996	8.4	17.8	8.1	412.6	7.2	56.6		
	23/09/1996	8.4	13.6	6.0	418.6	5.1	61.7		
pred-L4	24/09/1996	3.7	17.5	6.3	424.8	5.8	67.5		
	25/09/1996	5.2	17.3	6.3	431.1	6.6	6.6	DZ= 4.70	
	26/09/1996	10.0	14.0	7.0	438.1	7.3	13.9		
	27/09/1996	5.0	12.0	3.5	441.6	3.8	17.7		
	28/09/1996	5.3	12.0	3.7	445.2	4.0	21.6		
	29/09/1996	7.4	11.5	4.5	449.7	4.8	26.4		
	30/09/1996	9.3	19.5	9.4	459.1	9.7	36.1		
	1/10/1996	11.0	14.9	8.0	467.0	8.3	44.3		
	2/10/1996	8.5	16.0	7.3	474.3	7.6	51.9		
	3/10/1996	8.4	17.3	7.9	482.1	8.2	60.0		
pred-L5	4/10/1996	7.6	20.9	9.3	491.4	9.6	69.6		
	5/10/1996	10.3	15.5	7.9	499.3	8.2	77.8		
	6/10/1996	5.0	15.8	5.4	504.7	5.7	83.5		
	7/10/1996	6.3	13.4	4.9	509.5	5.2	88.6		
pred-L5	8/10/1996	5.2	19.4	7.3	516.8	7.6	96.2		
	9/10/1996	10.2	20.1	10.2	527.0	12.8	12.8	DZ= 2.40	
	10/10/1996	10.7	17.7	9.2	536.2	11.8	24.6		
	11/10/1996	9.2	11.5	5.4	541.5	8.0	32.5		

	12/10/1996	8.4	16.1	7.3	548.8	9.9	42.4		
	13/10/1996	9.4	18.6	9.0	557.8	11.6	54.0		
	14/10/1996	10.9	20.0	10.5	568.2	13.1	67.0		
	15/10/1996	9.6	24.4	12.0	580.2	14.6	81.6		
	16/10/1996	11.6	20.5	11.1	591.3	13.7	95.3		
	17/10/1996	8.0	17.3	7.7	598.9	10.3	105.5		
	18/10/1996	11.8	18.4	10.1	609.0	12.7	118.2		
	19/10/1996	10.7	14.6	7.7	616.7	10.3	128.5		
	20/10/1996	5.4	13.7	4.6	621.2	7.2	135.6		
	21/10/1996	5.9	17.7	6.8	628.0	9.4	145.0		
	22/10/1996	8.3	15.2	6.8	634.8	9.4	154.4		
	23/10/1996	8.7	25.4	11.9	646.6	14.5	168.8		
	24/10/1996	14.7	30.7	14.9	661.5	17.5	186.3		
pred-PP	25/10/1996	10.9	14.0	7.5	668.9	10.1	196.3		
	26/10/1996	5.2	13.0	4.1	673.0	6.7	203.0		
pred-PP	27/10/1996	9.0	18.2	8.6	681.6	11.2	214.2		
	28/10/1996	9.1	17.4	8.3	689.9	10.2	10.2	DZ= 3.01	
	29/10/1996	9.8	16.6	8.2	698.1	10.2	20.4		
	30/10/1996	3.9	14.8	4.9	703.0	6.3	26.8		
	31/10/1996	6.2	18.3	7.3	710.2	9.2	36.0		
	1/11/1996	6.4	20.4	8.4	718.6	10.4	46.4		
	2/11/1996	10.2	26.6	12.6	731.2	14.6	61.0		
	3/11/1996	13.9	19.9	11.9	743.1	13.9	74.9		
	4/11/1996	8.6	16.0	7.3	750.4	9.3	84.2		
	5/11/1996	6.9	16.5	6.7	757.1	8.7	92.9		
	6/11/1996	7.3	19.0	8.2	765.3	10.1	103.0		
	7/11/1996	11.4	17.3	9.4	774.6	11.3	114.3		
pred-P	8/11/1996	11.8	23.7	12.8	787.4	14.7	129.1		
pred-P	9/11/1996	11.2	18.5	9.9	797.2	11.8	140.9		
	10/11/1996	5.4	12.6	4.0	801.2				
	11/11/1996	6.2	16.3	6.3	807.5				
	12/11/1996	6.4	15.4	5.9	813.4				
	13/11/1996	7.4	22.2	9.8	823.2				
	14/11/1996	9.1	16.9	8.0	831.2				
	15/11/1996	6.5	13.2	4.9	836.0				
	16/11/1996	6.7	12.8	4.8	840.8				
	17/11/1996	7.5	13.0	5.3	846.0				
obs- PP	18/11/1996	6.5	13.2	4.9	850.9				
n=14									

1. Using fixed threshold of 5.00 °C, Predicted hatch = 171.29 DD > 5.00 °C.
Predicted hatch date (pred-L1) = 6 August 1996.
Observed hatch date (L1-hatch) = 12 August 1996.
Observed hatch was 6 days later at 204.10 DD (19.2% more than predicted).
2. Using fixed threshold of 5.00 °C, Predicted pre-pupa = 663.45 DD > 5.00 °C.
Predicted PP date = 25 October 1996.
Observed PP date = 18 November 1996.
Observed PP date = 24 days later at 850.85 DD (28.2% more than predicted).
3. Using egg threshold of 5.87 °C, Predicted hatch = 160.95 DD > 5.87 °C.
Predicted hatch date = 11 August 1996.
Observed hatch date = 12 August 1996.
Observed hatch was 1 day later at 171.60 DD (6.6% more than predicted).
4. Using new threshold for each stage:
Predicted pre-pupa = 9 November 1996.
Observed pre-pupa = 18 November 1996.
Observed pre-pupa = 9 days later than predicted.

Field validation. Trial-3 at CRC. Eggs and larvae reared outside.									
If minimum temperature < lower threshold, minimum temperature set at lower threshold.									
If maximum temperature > 25°C, maximum temperature set at 25°C.									
Oviposition on 21st March 1997.				THERMAL ACCUMULATION > 21 MARCH 1997.					
				OVERALL		EGGS		TIME-TO-L4	
DD = (min + max)/2 - threshold				DD>5 °C		DD>5.87 °C		DD>5.69 °C	
		min	max	Daily	Total	Daily	Total	Daily	Total
Ovipos.	21/03/1997	10.8	15.1		0.0		0.0		0.0
	22/03/1997	7.3	21.3	9.3	9.3	8.43	8.4	8.6	8.6
	23/03/1997	9.6	20.3	10.0	19.3	9.08	17.5	9.3	17.9
	24/03/1997	12.1	19.9	11.0	30.3	10.13	27.6	10.3	28.2
	25/03/1997	9.8	17.6	8.7	39.0	7.83	35.5	8.0	36.2
	26/03/1997	11.0	22.9	12.0	50.9	11.08	46.6	11.3	47.4
	27/03/1997	12.8	24.8	13.8	64.7	12.93	59.5	13.1	60.5
	28/03/1997	11.0	12.1	6.6	71.3	5.68	65.2	5.9	66.4
	29/03/1997	7.5	18.7	8.1	79.4	7.23	72.4	7.4	73.8
	30/03/1997	10.4	15.6	8.0	87.4	7.13	79.5	7.3	81.1
	31/03/1997	7.6	13.8	5.7	93.1	4.83	84.4	5.0	86.1
	1/04/1997	9.3	14.3	6.8	99.9	5.93	90.3	6.1	92.2
	2/04/1997	8.5	17.5	8.0	107.9	7.13	97.4	7.3	99.5
	3/04/1997	9.0	18.3	8.7	116.5	7.78	105.2	8.0	107.4
	4/04/1997	12.8	20.8	11.8	128.3	10.93	116.1	11.1	118.5
	5/04/1997	11.3	16.9	9.1	137.4	8.23	124.4	8.4	126.9
	6/04/1997	11.3	16.2	8.8	146.2	7.88	132.2	8.1	135.0
	7/04/1997	4.1	12.6	3.8	150.0	3.365	135.6	3.5	138.4
	8/04/1997	7.2	16.7	7.0	156.9	6.08	141.7	6.3	144.7
	9/04/1997	10.9	16.6	8.8	165.7	7.88	149.6	8.1	152.7
pred-L1	10/04/1997	5.5	16.0	5.8	171.4	5.065	154.6	5.2	157.9
	11/04/1997	5.0	16.1	5.6	177.0	5.115	159.7	5.2	163.1
pred-L1	12/04/1997	7.5	17.5	7.5	184.5	6.63	166.4	6.8	169.9
	13/04/1997	8.6	18.1	8.4	192.8	7.48	173.8	7.7	177.5
	14/04/1997	11.0	17.8	9.4	202.2	8.53	182.4	8.7	186.2
	15/04/1997	5.5	14.5	5.0	207.2	4.315	186.7	4.4	190.6
L1-hatch	16/04/1997	6.5	16.6	6.6	213.8	5.68	192.4	5.9	196.5
	17/04/1997	11.8	17.8	9.8	223.6			9.1	205.6
	18/04/1997	6.2	11.6	3.9	227.5			3.2	208.8
	19/04/1997	3.6	12.6	3.8	231.3			3.5	212.2
	20/04/1997	7.3	20.8	9.1	240.3			8.4	220.6
	21/04/1997	11.0	19.6	10.3	250.6			9.6	230.2
	22/04/1997	8.6	15.3	7.0	257.6			6.3	236.4
pred-L2	23/04/1997	5.3	19.8	7.6	265.1			7.1	243.5
	24/04/1997	9.2	12.8	6.0	271.1			5.3	248.8
	25/04/1997	1.8	16.1	5.6	276.7			5.2	254.0
	26/04/1997	5.6	19.2	7.4	284.1			6.8	260.7
	27/04/1997	15.5	24.4	15.0	299.0			14.3	275.0
	28/04/1997	13.7	23.1	13.4	312.4			12.7	287.7
pred-L3	29/04/1997	14.1	24.0	14.1	326.5			13.4	301.0
	30/04/1997	11.9	20.3	11.1	337.6			10.4	311.4
	1/05/1997	14.5	25.7	14.8	352.3			14.1	325.5
	2/05/1997	14.2	14.3	9.3	361.6			8.6	334.0
	3/05/1997	11.5	17.4	9.5	371.0			8.8	342.8
	4/05/1997	12.9	20.2	11.6	382.6			10.9	353.6
	5/05/1997	7.9	15.2	6.6	389.1			5.9	359.5
pred-L4	6/05/1997	5.4	17.4	6.4	395.5			5.9	365.3
	7/05/1997	7.3	11.9	4.6	400.1			3.9	369.2
pred-L4	8/05/1997	6.9	13.2	5.1	405.2			4.4	373.6
	9/05/1997	5.7	11.5	3.6	408.8			2.9	376.5
	10/05/1997	3.7	12.2	3.6	412.4			3.3	379.7

	11/05/1997	5.9	11.5	3.7	416.1			3.0	382.7
	12/05/1997	6.3	14.4	5.4	421.4			4.7	387.4
	13/05/1997	9.3	15.9	7.6	429.0			6.9	394.3
	14/05/1997	5.3	15.9	5.6	434.6			5.1	399.4
few L4	15/05/1997	6.1	13.8	5.0	439.6			4.3	403.6
	16/05/1997	8.1	11.7	4.9	444.5			4.2	407.8
	17/05/1997	3.3	13.6	4.3	448.8			4.0	411.8
	18/05/1997	4.9	13.9	4.5	453.2			4.1	415.9
	19/05/1997	4.0	13.3	4.2	457.4			3.8	419.7
~ half L4	20/05/1997	7.1	11.5	4.3	461.7			3.6	423.3
	21/05/1997	3.2	13.1	4.1	465.7				
	22/05/1997	4.9	12.5	3.8	469.5				
	23/05/1997	8.9	12.1	5.5	475.0				
	24/05/1997	4.7	13.0	4.0	479.0				
	25/05/1997	3.5	12.8	3.9	482.9				
pred-L5	26/05/1997	4.3	15.5	5.3	488.1				
	27/05/1997	6.6	18.5	7.6	495.7				
	28/05/1997	11.5	19.1	10.3	506.0				
	29/05/1997	5.2	10.0	2.6	508.6				
	30/05/1997	4.7	11.0	3.0	511.6				
obs-L5	31/05/1997	2.9	12.4	3.7	515.3				
	1/06/1997	3.3	12.4	3.7	519.0				
	2/06/1997	3.6	10.5	2.8	521.7				
	3/06/1997	3.2	13.7	4.4	526.1				
	4/06/1997	2.6	13.6	4.3	530.4				
	5/06/1997	4.1	16.8	5.9	536.3				
	6/06/1997	6.6	16.6	6.6	542.9				
	7/06/1997	6.5	13.5	5.0	547.9				
	8/06/1997	4.7	14.3	4.7	552.5				
	9/06/1997	6.6	12.2	4.4	556.9				
	10/06/1997	4.4	10.5	2.8	559.7				
	11/06/1997	6.0	16.0	6.0	565.7				
	12/06/1997	8.2	14.1	6.2	571.8				
	13/06/1997	9.2	16.5	7.9	579.7				
	14/06/1997	5.7	10.8	3.3	582.9				
	15/06/1997	4.0	13.1	4.1	587.0				
	16/06/1997	5.5	13.5	4.5	591.5				
	17/06/1997	4.2	12.4	3.7	595.2				
	18/06/1997	6.1	11.6	3.9	599.0				
	19/06/1997	7.4	10.3	3.9	602.9				
	20/06/1997	2.6	11.0	3.0	605.9				
	21/06/1997	2.0	10.5	2.8	608.6				
	22/06/1997	2.5	11.1	3.1	611.7				
	23/06/1997	4.4	12.2	3.6	615.3				
	24/06/1997	6.2	10.3	3.3	618.5				
	25/06/1997	5.4	9.9	2.7	621.2				
	26/06/1997	4.7	11.0	3.0	624.2				
	27/06/1997	6.0	11.2	3.6	627.8				
	28/06/1997	4.9	12.1	3.6	631.3				
	29/06/1997	4.2	10.3	2.7	634.0				
	30/06/1997	2.3	10.6	2.8	636.8				
into LAB	1/07/1997	3.5	10.9	10.0	646.8				
	2/07/1997	2.4	13.3	10.0	656.8				
pred-PP	3/07/1997	4.4	9.3	10.0	666.8				
	4/07/1997	4.0	9.4	10.0	676.8				
	5/07/1997	5.2	11.6	10.0	686.8				
	6/07/1997	4.4	11.9	10.0	696.8				
	7/07/1997	5.3	13.5	10.0	706.8				
	8/07/1997	6.1	16.4	10.0	716.8				

	9/07/1997	1.9	5.7	10.0	726.8				
	10/07/1997	1.8	10.9	10.0	736.8				
	11/07/1997	4.2	11.9	10.0	746.8				
	12/07/1997	6.9	12.9	10.0	756.8				
	13/07/1997	6.6	13.7	10.0	766.8				
	14/07/1997	5.3	13.8	10.0	776.8				
Pupae	15/07/1997	6.8	13.7	10.0	**786.8**	**pred-pup**			
1. Using fixed threshold of 5.00 °C, Predicted hatch = 171.29 DD > 5.00 °C.									
Predicted hatch date (pred-L1) = 10 April 1997.									
Observed hatch date (L1-hatch) = 16 April 1997.									
Observed hatch was 6 days later or 42.46 DD (24.8%) more than predicted.									
2. Using fixed threshold of 5.00 °C, Predicted pupae = 784.03 DD > 5.00 °C.									
Predicted Pupae date = 15 July 1997.									
Observed mid-point of pupation = 15 July 1997.									
Observed pupation consistent with predictions.									
3. Using egg threshold of 5.87 °C, Predicted hatch = 160.95 DD > 5.87 °C.									
Predicted hatch date = 12 April 1997.									
Observed hatch date = 16 April 1997.									
Observed hatch was 4 days later at 192.37 DD (19.5% more than predicted).									
4. Using threshold of 5.7 °C for Time-to-L4, predicted L4 = 371.66 DD > 5.7 °C									
Predicted L4 = 8 May 1997.									
Observed L4 = 20 May 1997.									
Observed L4 = 12 days later at 423.25 DD (13.9% more than predicted).									
5. Using threshold of 5.0 °C for Time-to-L4, predicted L4 = 390.38 DD > 5.0 °C									
Predicted L4 = 6 May 1997.									
Observed L4 = 20 May 1997.									
Observed L4 = 14 days later at 461.7 DD (18.3% more than predicted).									

APPENDIX C

Isozyme Analysis of *M. privata* populations.

C.1 AIMS:

1. To compare geographically similar populations with different phenologies.

It is proposed to first compare high and low elevation populations within the Derwent River valley which have contrasting seasonal phenologies. The null hypothesis is that there will be no significant difference in the mean frequency of rare alleles between the different temporal populations. Currently, I have 100 larvae per population from two populations from both high and low elevation areas, a total of 400 larvae. This will allow a comparison of different populations within each region. It is possible that each population will be genetically distinct, possibly influenced by founder effects from few individuals. There may be as much variation between different low-elevation populations as between high- and low- elevations.

2. To compare geographically different populations which have similar phenologies.

The next comparison proposed is of different geographic populations in southern Australia which all have a similar seasonal phenology. The null hypothesis is that there will be no significant difference in the mean frequency of rare alleles between the different geographic populations. Currently, I have between 30 and 50 larvae per population from one WA and three Victorian populations. It will be interesting to compare the variation between spatially different populations with that between temporally different populations.

C.2 METHODS:

C.2.1 Insect collection and storage

Larvae were collected between May and August 1997 from different areas according to the seasonal phenology at the different areas. Larvae collected during May were from summer populations at high elevation sites (600m) in central Tasmania. Oviposition at those sites usually begins in early January, and ends by late February. Larvae collected during July and August were from winter populations elsewhere. Peak oviposition at the latter sites is usually in March and April. All larvae were snap frozen in liquid nitrogen, then stored at -80°C until izozyme analysis.

C.2.2 Enzymes

Pupae were used during a pilot study which screened a total of 28 enzymes in three different buffer systems: morpholine citrate (MC), lithium borate (Li), and histidine (Hist.), although not all enzymes were tested in all three buffer systems. Eight enzymes were identified as potentially scorable:

Lithium Hydroxide buffer system:

1. Aspartate aminotransferase (AAT) E.C. Number 2.6.1.1
(also known as Glutamate-oxaloacetate transaminase (GOT))
2. α-Esterase (ES) E.C. Number 3.1.1.1

Histidine buffer system:

3. 6-Phosphogluconate Dehydrogenase (6PGDH) E.C. Number 1.1.1.44
4. Phosphoglucomutase (PGM) E.C. Number 2.7.5.1
5. (FK) E.C. Number
6. (LAP) E.C. Number
7. Glyceraldehyde-3-Phosphate Dehydrogenase (GA3PD) E.C. Number 1.2.1.12
8. Glycerol-3-Phosphate Dehydrogenase (GPD) E.C. Number 1.1.1.8

Staining quality was generally superior in histidine, and poor in MC and Li, except for the enzymes AAT and EST in Li mentioned above. The latter two enzymes were not tested in Hist., but should be to determine whether all eight enzymes might be screened by a single buffer system.

C.2.3 Sample preparation:

Live, or freshly thawed frozen material was crushed in an equal volume of extraction buffer. Extraction buffer used was 0.1M Tris Maleate (pH 8.0). To 10ml of buffer, 10 µl β-Mercaptoethanol and 20 µl Triton X-100 (1:10 dilution) were added. Ground samples were kept on ice until all samples were prepared. Paper wicks were used to load samples into gels.

Li OH gels were run at 280V constant.

Histidine gels were initially run at 280V constant, but this was recently reduced to 200V.

Table C- 1 Enzyme screening results for autumn gum moth pupae in three gel systems.

Gel	Enzyme	Stain	Quality	No. Loci	No. Alleles	Dist.(mm)	Comments
MC	IDH	Yes	-	1	2 ?		-
(C)	ADH	x	-	-	-		-
morpholine	SKDH	x	-	-	-		-
citrate	MDH	Yes	-	1	2 ?		-
	GPI(PGI)	Yes	-	nr	nr		monomorphic
	UGP	Yes	light	nr	nr		monomorphic
	PGD	x	-	-	-		-
	PGM	Yes	good	1	2	13,16	-
	LDH	Yes	faint	1	-	5	small movement
	FDH	x	-	-	-		-
	SOD	x	-	-	-		-
	PRO	Yes	fair		1 band	2	small movement
	ACP	Yes	smeared	1	2?	3,7	-
	G-6PDH	Yes	weak	1	1	5	mono; poor
	ME	Yes	smeared, heavy	1	1	19	mono; poor
	LAP	Yes	weak	2?	1	7	streaked, poor
					1	13	
	FUM	x	-	-	-	-	poor
	GA3PD	Yes	mod		1	3	mono; (use hist.)
	HK	Yes	weak	1	1	15	poor, not scorable
	GPD	Yes	good		1,2?	8	better in hist.
Li Borate	GDH	x	-	-	-		-
(standard)	AAT	Yes	-	1	-		looks variable
	AP(ACP)	Yes	smeared	1 ?	-		TRY AGAIN
	EST	Yes	-	2 strong	-		-
	MPI	Yes	weak	-	4	55,62,63,64	-
	ME	Yes	weak	-	2	20,25	TRY AGAIN
	SOD	x	-	-	-		-
	PEP	Yes	smeared	-	-	42-45	wrong colour
	PGM	Yes	weak	-	3	30,35,38	stain >1hr old
	LDH	x	-	-	-		TRY AGAIN
	FDH	Yes	weak	-	1	20	TRY AGAIN
	PRO	Yes	-	-	2 bands	19,24	-
	G-6PDH	Yes	streaked	?	?	2-15, 15-35	
	TPI	x	trace	-	-	26	poor; not scorable
	ALDO	Yes	v. weak	2?	1,1	17,30	poor
	FK	x	-	-	-	-	-
	LAP	Yes	streaked	-	-	-	poor
	FUM	Yes	weak	1	1	27	poor
	HK	Yes	weak	1	1	28	poor; not scorable
	GPD	Yes	weak	1	1	23	poor; mono
Histidine	6PGDH	Yes	good	1	2?	11,13	-
	ACP	Yes	smeared	-	-	10-28	Try C &Li
	PGM	Yes	good	-	3	10-26	heavy stain
	LDH	Yes	-	-	1	10	small movement
	G6PDH	Yes	good	-	1 or 2	5	Try C &Li
	PRO	Yes	good	-	2 bands	6,10	
	LDH	x	-	-	-	-	-
	FDH	x	-	-	-	-	-
	ALDO	Yes	mod	1	1	6	mono
	FK	Yes	weak	3?	1,3,1	28,17-21,10	poly; good; repeat
	LAP	Yes	good	2	1	26-30	1st loci good
					2,3?	16-20	
	FUM	x	-	-	-	-	poor
	GA3PD	Yes	good	1	2	4,6	scores well
	GPD	Yes	good	1	1?	18-21	mono